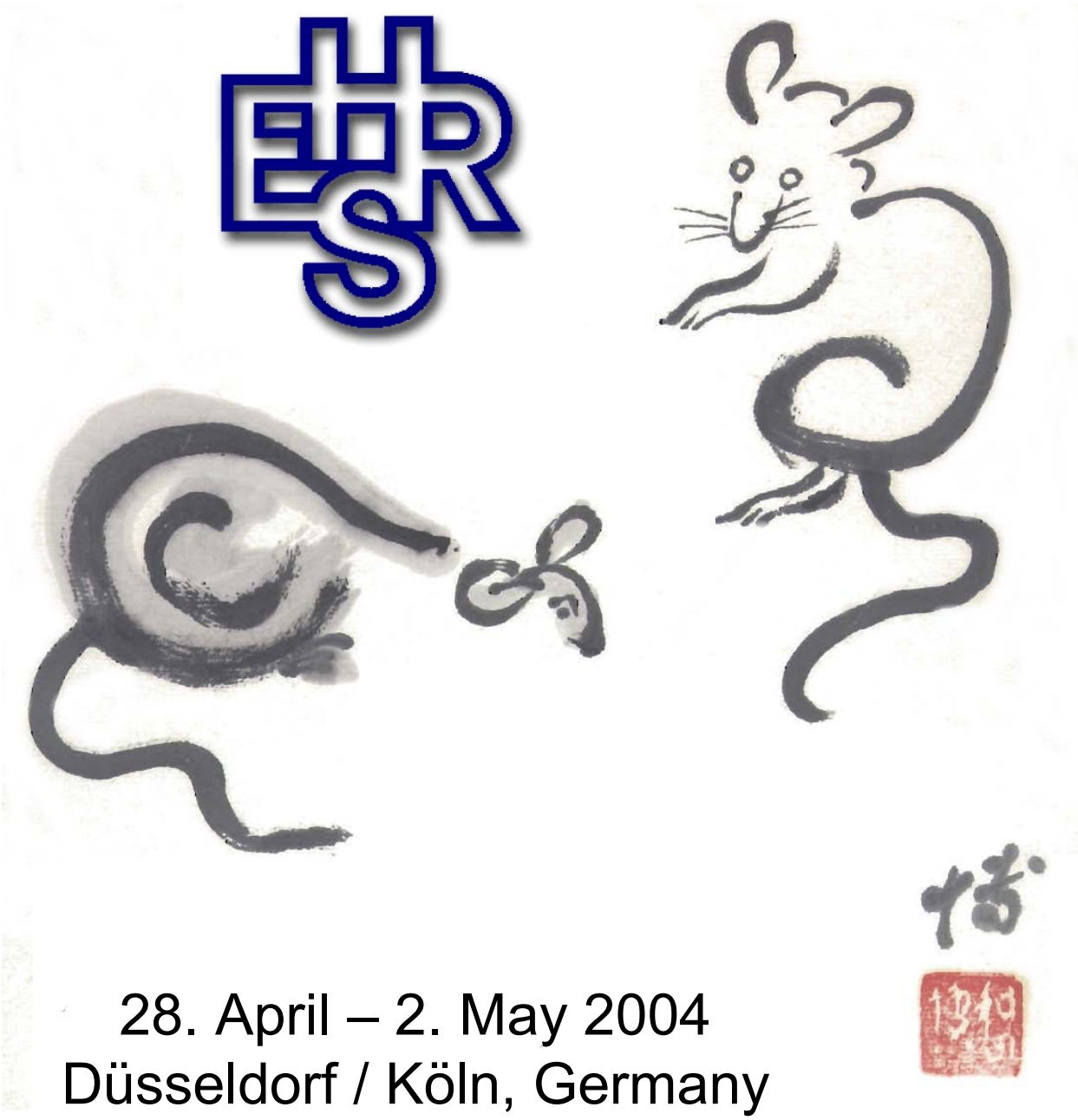
33. Annual Meeting European Histamine Research Society



28. April – 2. May 2004
Düsseldorf / Köln, Germany

HEINRICH HEINE
UNIVERSITÄT
DÜSSELDORF

Dear Histaminologists 28. April 2004

Welcome to the 33rd Annual Meeting of the European Histamine Research Society at the Kardinal Schulte Haus near Cologne. Many of you have seen Cologne at the occasion of the 1993 meeting. The host institution is now located 30 km to the north: Department of Neurophysiology, Heinrich-Heine-University, Düsseldorf. Heinrich Heine, the patron of our University and one of our major poets, was born in Düsseldorf in 1797, he died in Paris in 1856. He wrote romantic poems often with a unique and unusual ironic or disillusioning twist at the end. He was the founder of the modern feuilleton and wrote a bit caustic about his birthplace. Nevertheless, Düsseldorf is worth a visit, there is interesting recent architecture (e.g. O'Gehry) a fine art collection (20th century) and a jewel in the south: Benrath castle and park (1777).

We meet at the south end of "Bergisches Land" that we will explore during our excursion. The chemical industry founded by Bayer and Leverkus is around us: Leverkusen and Wuppertal. Düsseldorf is a village (1/2 Mio inhabitants) located at the mouth of the river Düssel into the Rhine. A formerly romantic part of the Düssel-valley has been praised by the 17th century pastor Neander and is consequently called the Neanderthal, the valley where the first bones of the Neandertal-man were found in 1856. Schloss Burg is a mediaeval castle, its 12th century appearance has been restored and a museum recalls the long bygone times. We will have dinner there. A historic iron bridge (Müngstener Brücke) spans the nearby valley of the river Wupper.

We are at home in quite diverse fields of biomedical research and assemble again to discuss the news. Thank you for the interesting abstracts promising a very attractive scientific program consisting of oral and poster presentations. There is no "prestige"-difference between these two. Some data are better presented this and some the other way. Most preferences have been approved by the abstract evaluation committee. Brief presentations in front of the posters are expected. Our main lectures also cover the spectrum of histamine research. Three of our symposia are dedicated to scientists and friends who recently deceased but left remarkable traces in our field and society: Czeslaw Maslinski, Börje Uvnäs and Hiroshi Wada.

Helmut Haas
Chair Organizing Committee
Department of Neurophysiology

cover design with a drawing by Hiroshi Wada

XXXIII Meeting of the EHRS
28th April – 2nd May 2004

Organizing Committee

Helmut Haas
Madeleine Ennis
Anneliese Voss
Aisa Chepkova
Tatjana Korotkova
Oliver Selbach
Olga Sergeeva

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Madeleine Ennis (Chairman)
Patrizio Blandina
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Tatjana Irman Florjanc
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Poster Prize Committee

K Rimvall (Chair)
E Assem, R Cacabelos, F Diel, B Gibbs,
N Grosman, E Masini, P Panula, B Passani
W Schunack, C Tiligada, K Yanai

XXXIII Meeting of the EHRS

28th April – 2nd May 2004

General Information

Location of the meeting:

Kardinal Schulte Haus (KSH, Thomas Morus Akademie)

Overather Str. 51 –53 Tel ++49 2204 4080
51429 Bergisch-Gladbach Fax ++49 2204 408697

Local access by public transport from Köln (Cologne) main station:

1. Busbahnhof Breslauer Platz (Bus-Station at the back of the train station). Schnellbus (fast bus) No 31, to Thomas-Morus-Akademie (ca. 30 min, stops at the entrance-arch of KSH).
2. S-Bahn to Bergisch-Gladbach, then the bus direction Overath or Moitzfeld until Bensberg.

Host

Helmut Haas
Department of Neurophysiology 22.03.01
Heinrich-Heine-Universität, Universitätsklinikum Düsseldorf
POB 101007, D – 40001 Düsseldorf
Phone ++49 211 811 2687 Fax ++49 211 811 4231

Registration desc open

Wednesday 28th April	15:00 – 20:00
Thursday 29th April – Saturday 1st May	08:00 – 12:00
Sunday 2nd May	08:00 – 09:00

Posters

will be on show during the whole meeting. Guided tours will be organized so that each poster presenter has the opportunity to give a 1 to 3 min explanation. Posters are “vertically” oriented 90 cm width 120 cm height. Poster prizes will be presented at the farewell dinner.

Social events and excursion

28th. April	20:15	Get together
29th April		Accompanying persons programme: visiting Cologne
	18:15	Organ Concert
30th April	13:00 – 23:00	Excursion to the Neandert al, Visit to the Museum and the place where the first Neanderthal bones were found, Dinner in “Schloss Burg”, a mediaeval castle.
1st May	19:00	Farewell Dinner

Sponsors

Abbott Laboratories, Azwell, Bayer AG, Dainippon Pharmaceuticals, Eiken Chemical, Eisai, Glaxo Smith Kline, Hoffmann-LaRoche, Johnson and Johnson, Kyowa Hakko Kogyo, Novo Nordisk A/S, Pfizer, UCB
Heinrich-Heine-Universität Düsseldorf and
Freunde und Förderer der Heinrich-Heine-Universität Düsseldorf

XXXIII Meeting of the EHRS
28th April – 2nd May 2004

The programme at a glance

Wednesday 28th April

15:00 – 20:00		Registration
16:00		EHRS council meeting
19:00		Opening Ceremony
19:30		JS Lin Opening lecture
20:15		Get together with Buffet

Thursday 29th April

08:30	L 2	C Teuscher main lecture
09:15	O 1	Czeslaw Maslinski Symposium
10:25	P 1 / 2	Poster discussion
11:30	O 2	H3-antagonists
13:30	O 3	Nervous system
14:45	P 3 / 4	Poster discussion
15:30	O 4	Clinical implications

Friday 30th April

08:30	L3	P Mannaioni GB West lecture
09:15	O 5	Börje Uvnäs Symposium
10:30	O 6	Young investigators award symposium
11:30	P 5 / 6	Poster discussion
13:00		Excursion www.neanderthal.de/ www.schlossburg.de/

Saturday 1st May

09:00	L4	R Leurs main lecture
09:45	O 7	Histamine receptors
13:30	O8	Hiroshi Wada Symposium
16:00		General assembly of the EHRS
19:00		Farewell Dinner

Accompanying persons programme

Wednesday 28th April

20:15	Get together with Buffet

Thursday 29th April

09:00	Visit Köln (Cologne)
18:15	Organ Concert at KSH

Friday 30th April

13:00	Excursion Neanderthal

Saturday 1st May

19:00	Farewell Dinner

XXXIII Meeting of the EHRS
28th April – 2nd May 2004

Programme

Wednesday 28th April

15:00 – 20:00 Registration

16:00 EHRS council meeting

19:00 **Opening Ceremony**

Prof. Helmut Haas
Department of Neurophysiology, Heinrich-Heine-Universität, Düsseldorf

Prof. Jürgen Schrader
Rector for Research, Heinrich-Heine-University, Düsseldorf

Prof. Madeleine Ennis
President of the EHRS

Opening Lecture

19:30 **Jian-Sheng Lin**, Lyon, France
HISTAMINE AND OREXIN NEURONS: SYNERGISTIC AND COMPLEMENTARY REGULATION OF THE SLEEP-WAKE CYCLE?

20:15 Get together with Buffet

Thursday 29th April
morning

08:30 **Main lecture** Introduction: **H. Haas**

C Teuscher, Burlington, Vermont, USA

HISTAMINE RECEPTORS AND SUSCEPTIBILITY TO CENTRAL NERVOUS SYSTEM AUTOIMMUNE DISEASE

9.15 O 1 Czeslaw Maslinski Symposium

Chair: **W Schunack, W Schmutzler**

09:15 **W Schunack** Czeslaw Maslinski – A founder member of the Histamine Club

09:25 **E Masini** HDC activity and histamine content correlate with Prostaglandin E_2 production and tumor stage in human colorectal cancer

09:40 **H Schwelberger** Characterization of four genes encoding porcine copper-containing amine oxidases

09:55 **N Huetz** Characterisation of native and recombinant histamine N-methyltransferase proteins

10:10 **A Fogel** Gastrointestinal histamine system in experimental ulcerative colitis in rats

10:25 **Coffee break and poster discussions 1 and 2**
(see pages 8,9)

11.30 O 2 H3 antagonists

Chair: **P Chatelain, N Carruthers**

11:30 **K Rimvall** Body-weight lowering effects of the cinnamic amide, NNC 0038-0000-1202, a novel histamine H3 receptor antagonist, in obese rodents

11:45 **A. Hancock** Antiobesity evaluation of histamine H3 receptor antagonist analogs of A-331440 with improved safety and efficacy

12:00 **M Cowart** Achievement of behavioral efficacy and improved potency in new heterocyclic analogs of Benzofuran H3 antagonists

12:15 **AD Medhurst** Pre-clinical evaluation of novel H3 receptor antagonists

12:30 **Lunch**

Thursday 29th April
afternoon

13.30 O 3 **Nervous system** Chair **J Huston, P Blandina**

13:30 **G Cenni** Cannabinoids modulate the activity of histaminergic neurons

13:45 **O Sergeeva** Epsilon subunit expression and propofol modulation of $GABA_A$ receptors in acutely isolated histaminergic neurons

14:00 **K Yanai** [^{11}C] Doxepin-PET study of H_1 receptors in psychiatric patients

14:15 **G Bongers** Modulation of Akt/GSK3-β axis as new signaling properties of the histamine H_3 receptor

14:30 **GB Fox** Selective H_3 receptor blockade: Broad efficacy in cognition and schizophrenia models

14:45 **Coffee break and poster discussions 3 and 4**
(see pages 10, 11)

16.30 O 4 **Clinical Implications** Chair: **A Falus, M Carman Krzan**

16:30 **H Hegyesi** Histamine mediated modulation of tumor progression marker expression in mice experimental dermatofibrosarcoma model

16:45 **H Ohtsu** The curative effect of histamine on cutaneous wound healing process

17:00 **B Horr** Expression and cleavage of STAT1alpha is regulated via histamine (H4) receptor signalling expression and cleavage

17:15 **R Khanferyan** The influence of histamine H3 receptor antagonists on IgE synthesis

17:30 **J Klocker** Plasma diamine oxidase during continuous administration of heparin in thrombosis patients

18:15 **Organ concert (Bach, Buxtehude)**

19:15 **Dinner**

Poster and Discussion Sessions **Thursday 29th April**
morning

Guided tours with 1-3 min oral presentations

11:15 **P 1** Chair: **C Tiligada, F Diel**

P1-1 HYA Lau Inhibition of mast cell histamine release by specific phosphodiesterase inhibitors

P1-2 A Byrne A study on the mast cell stabilising properties of *Sargentodoxa cuneata*

P1-3 FH Falcone Lack of Protease Activated Receptor (PAR) expression in purified human basophils

P1-4 N Strenzke Pharmacological studies on the role of reactive oxygen species in IgE-dependent histamine secretion from human basophils

P1-5 Z Wiener Interleukin (IL)-9 increases the expression of several cytokines in activated mast cells and the IL9-induced IL-9 production is inhibited in mast cells of histamine-free transgenic mice

P1-6 V Wilken Double-blind, placebo-controlled oral provocation with histamine revealed placebo-associated histamine release in mastocytosis (M) but not in controls

P1-7 A Davidsson Histamine release from blood cells and serum ECP in patients with asthma, during and after a mild pollen season.

P1-8 L Rohn Can Histamine be tasted in wine?

P1-9 U Mohar Comparing two different methods on two isolated preparations for estimation of antagonist potency

Poster and Discussion Sessions **Thursday 29th April**
morning

Guided tours with 1-3 min oral presentations

11:15 **P 2** Chair: **B. Passani, K. Yanai**

P2-1 N Doreulee Histamine-evoked cortico-striatal synaptic plasticity and short-term-potentiation are impaired in rats with porto-caval anastomosis

P2-2 TK Kukko-Lukjanov The neuroprotective effect of the central histaminergic neuron system on kainic acid-induced neuronal death in the developing hippocampus in vitro

P2-3 M Lintunen Histamine, a bad boy or a good girl in kainic acid-induced epilepsy?

P2-4 IS Midzyanovska Distribution of the H1-histamine receptor in the brain regions of epileptic and normal rats

P2-5 D Maslinska Post-infectional distribution and phenotype of mast cells penetrating human brains

P2-6 N Fernandez Histamine H2 receptor regulates GRK2 expression by a dual mechanism in U-937 cell line

P2-7 S Kakavas Histamine levels in the rat hypothalamus under pathological conditions

P2-8 S Rajtar The influence of amitriptyline and sertraline in vitro on the kinetics of exogenous histamine in cat blood

P2-9 S Bour Tyramine and benzylamine but not histamine partially mimic the adipogenic effect of insulin in a human preadipocyte cell strain

P2-10 M Lipnik-Stangelj Histamine and IL-6 interactions in the stimulation of nerve growth factor secretion from cultured astrocytes

P2-11 R Nosal Antiplatelet and antiphagocyte activity of H_1-antihistamines

P2-12 K Tekes Effects of nociceptin on neuronal and mast cell histamine in the brain.

Poster and Discussion Sessions **Thursday 29th April afternoon**

Guided tours with 1-3 min oral presentations

14.45 **P 3** Chair: **H Ohtsu, N Grosman**

P3-1 D Szukiewicz Mast cell-derived VEGF and VEGF receptor type 1, 2, and 3 expression in human term trophoblast culture – influence of hypoxia.

P3-2 G Szewczyk Influence of histamine on the process of human trophoblast differentiation

P3-3 WA Fogel Ornithine and histidine decarboxylases in hypertrophic and hyperplastic mouse kidney

P3-4 J Klocker Expression of histamine degrading enzymes in porcine tissues

P3-5 J Petersen Characterization of functional polymorphisms of the human diamine oxidase gene

P3-6 HW Schultis Tandem mass spectroscopy as a new reliable and sensitive method for the detection of n-tele methylhistamine (MH) in urine

P3-7 K Knies Effects of moderate stress on plasma histamine levels in laboratory dogs

P3-8 F Ahrens Plasma histamine levels in rescue dogs under different training conditions

Poster and Discussion Sessions — Thursday 29th April afternoon

Guided tours with 1-3 min oral presentations

14.45 **P 4** Chair: **P Panula, R Cacabelos**

P4-1 E Dere Reduced open-field exploration, increased anxiety and improved rotarod performance concomitant with alterations in brain acetylcholine and monoamine systems after histidine-decarboxylase gene knockout in 129/Sv mice

P4-2 M Efoudebe Improvement in spatial memory by amthamine, a histamine H2-receptor agonist, requires hippocampal ERK activation

P4-3 D Unterbrink Histaminergic modulation of the hippocampal and striatal EEG oscillatory profile in the behaving rat as revealed by Wavelet Feature Extraction

P4-4 KS Eriksson Orexin / dynorphin neurons control GABAergic inputs to histaminergic neurons

P4-5 DR Stevens Alpha2-Adrenergic receptor-mediated presynaptic inhibition of GABAergic IPSPs in rat histaminergic neurons

P4-6 TM Korotkova Histamine excites noradrenergic neurons in locus coeruleus in rats

P4-7 T Okuda The effects of H1 antagonists on the methamphetamine-induced psychomotor activation in rats: The correlation to tissue methamphetamine concentrations

P4-8 M Kopilov Dual effects of pyrilamine on the absence seizures in the WAG/Rij rats

P4-9 G Gisselmann Primary structure and functional expression of two histamine-gated ion channels in *Drosophila melanogaster*

Friday 30th April

GB West Lecture Introduction: **E Masini**

08:30 **P Mannaioni,** Firenze, Italy
THE RIDDLE OF MAST CELL RECEPTORS
IN THE IMMUNE RESPONSE

09.15 **O 5** **Börje Uvnäs-Symposium** Chair: **A Sydbom, M Ennis**

9:15 **A Sydbom** Börje Uvnäs – a pioneer in mast cell research

9:25 **ESK Assem** Effect of cyclosporin A on secretion from intact and permeabilized mast cells

9:40 **M Ennis** Mast cell mediator release after endobronchial challenge with AMP in normal subjects

9:55 **RL Thurmond** Antagonists of the histamine H4 receptor

10:10 Coffee

10.30 **O 6** **Young Investigator's Award Symposium**
Chair **A Hancock, A Fogel**

10:30 **T Ishizuka** Evidence for histaminergic involvement in the action of modafinil

10:45 **SA Mätzler** Expression of copper amine oxidases in porcine tissues

11:00 **F Shenton** Human H_3 histamine receptor isoforms can form homooligomers

11:15 **A Sponring** Characterization of the porcine diamine oxidase gene promoter

11:30 - 12:45 **Poster discussions 5 and 6** (see pages 13, 14)

13:00 **Excursion Neanderthal**

Dinner at Schloss Burg

ca. 23:00 **back at Kardinal-Schulte-Haus (KSH, meeting site)**

Poster and Discussion Sessions — Friday 30th April morning

Guided tours with 1-3 min oral presentations

11.30 **P 5** Chair: **ESK Assem, BF Gibbs**

P5-1 AK Fulop Tissue specific alteration of glucocorticoid receptor number in histamine free mice

P5-2 E Pap Histamine deficiency alters the testosterone production of Leydig cells in primary culture

P5-3 H Borck Histamine delays STAT6 phosphorylation in atopic human lymphocyte cell cultures

P5-4 VA Medina Histamine is a selective protector against cellular damaged produced by ionizing radiation

P5-5 L Uberti Effect of H_3 receptor ligands on ethanol induced damage in rat gastric mucosal cells

P5-6 LC Tetlow The effects of histamine on rheumatoid synovial fibroblasts in vitro: a comparison with human articular chondrocytes

P5-7 DE Woolley Immunolocalisation of histamine H1 and H2 receptors in cells and tissues from osteoarthritic and rheumatoid joints

P5-8 E Wojtecka-Lukasik Taurine chloramine modifies the development of adjuvant-induced arthritis

P5-9 E Wojtecka-Lukasik Is estimation of lymphocyte histamine content useful in the identification of nonresponders among rheumatoid arthritis (RA) patients treated with anti-TNF-alfa monoclonal antibodies ?

P5-10 BYC Wan Effects of genistein on rat ileum smooth muscle contraction

P5-11 G Ciuzynska The influence of 5-HT_2 and 5-HT_3 receptor agonists on the haemodynamics of the isolated constant pressure perfused, rat heart

P5-12 D Szukiewicz TGF-β1-induced attenuation of isolated placental arteries response to histamine is stronger in preeclampsia

P5-13 J Jochem The role of peripherally acting histamine in metoprine-induced reversal of haemorrhagic shock in rat-skeletal muscle microcirculatory studies

Poster and Discussion Sessions — Friday 30th April morning

Guided tours with 1-3 min oral presentations

11.30 P 6 — Chair: **W Schunack, K Rimvall**

P6-1 H Fukui Histamine H1 receptor-mediated histamine H1R gene expression

P6-2 M Gillard Comparison of ex vivo and in vivo binding data applied to brain histamine H_1 receptor

P6-3 B Christophe Comparison of the receptor occupancy kinetics of levocetirizine at peripheral and centra H_1R after oral administration in the guinea pig

P6-4 A van Marle Characterization of fluorescent ligands for the histamine H_2 receptor

P6-5 B Peschke Cinnamic amides of (*S*)-2-(Aminomethyl)pyrrolidines – discovery and SAR of the potent H3-antagonist NNC 0038-0000-1202

P6-6 B Schlegel Molecular dynamics simulations of histamine H3-receptor / ligand complexes

P6-7 R Yoshimoto Complex pharmacology of GT-2331 in *in vitro* and *in vivo*

P6-8 R Kitbunnadaj Identification of 4-(1*H*-Imidazol-4-yl-methyl)pyridine (Immethridine) as a novel potent and highly selective histamine H_3 receptor agonist

P6-9 TA Esbenshade Use of novel, non-imidazole inverse agonist radioligands to define histamine H_3 receptor pharmacology

P6-10 RM van Rijn Homo-oligomerization of the human histamine H_4 receptor

P6-11 AF Lozada Expression of the histamine H4 receptor during rat development

P6-12 HD Lim Activity modulation of the human histamine H_4 receptor by hydrophobic group-containing ligands

P6-13 BT Amberger Single-cell RT-PCR analysis of adenosine deaminase expression in rat histaminergic neurons

Saturday 1st May 2004
morning

09:00 **Main Lecture** Introduction: **H Timmerman**

R Leurs Amsterdam
HOW TO BIND AND ACTIVATE HISTAMINE H1 RECEPTORS

09:45 **O 7** **Histamine receptors** Chair **H Timmerman, H Stark**

09:45 **H Stark** Further hints for protean agonism at histamine H_3 receptors with novel 4-(ω-(Alkyloxy)alkyl)-1*H*-imidazoles

10:00 **R Bakker** Homo-oligomerization of the human histamine H_1 receptor

10:15 **F Monczor** (GB West essay prize winner)
Mepyramine, an H1 inverse agonist, binds preferentially to a G protein coupled form of the receptor and sequesters G Protein

10:35 Coffee break

11:00 **NI Carruthers** (1-[4-(3-Piperidin-1-ylpropoxy)benzyl]piperidine): A template for the design of potent and selective non-imidazole histamine H3 receptor antagonists

11:15 **RG Booth** Ligand-directed multifunctional signaling of histamine H_1 receptors

11:30 **JV Fleming** The ~36kDa isoform of rat L-HDC is capable of dimerization but is nevertheless deficient in the binding and decarboxylation of substrate

11:45 **Final Poster viewing by Poster Jury**

12:30 **Lunch**

Saturday 1st May 2004
afternoon

13:30 O 8 Hiroshi Wada – Symposium

Chair: **T Watanabe, H Haas**

13:35 **H Haas** Introduction

13:40 **T Watanabe** Obituary – Prof. Hiroshi Wada

13:50 **P Panula** Association of altered brain histaminergic system and related genes with addictive behaviour and psychiatric diseases

14:15 **R Leurs** From rat liver [^{3}H]mepyramine binding to solid state NMR measurements of the histamine H_1 receptor

14:40 **R Cacabelos** The pathogenic role of histamine in Alzheimer disease

15:05 **H Fukui** Studies of histamine H1 receptor functions at molecular and physiological levels

15:30 Coffee break

16:00 General Assembly of the EHRS

19:00 Farewell Dinner

Abstracts

first authors in alphabetical order

Plasma histamine levels in rescue dogs under different training conditions

F. Ahrens[1], K. Knies, M. Schneider and M.H. Erhard

Institute of Animal Welfare, Ethology and Animal Hygiene, Faculty of Veterinary Medicine, Ludwig-Maximilians-University Munich.

An effective training of rescue dogs is the basis for a successful search of missing people in avalanches. Elevated levels of plasma histamine, either due to mechanical or thermic stimulation of mast cells, might indicate inadequate training conditions. Stress response should clearly refer to an overloaded and thereby ineffective training of the dogs. Therefore, in this study we investigated levels of plasma histamine and cortisol in rescue dogs under different conditions.

Sixteen rescue dogs underwent four different types of strain (running and searching, each in summer and winter, 700 and 2600 metres above sea-level, respectively) with a duration of 2x20 min each and a 20 min break in between. Outdoor temperature ranged between 9 and 26 degrees in summer and –17 and –3 degrees Celsius in winter. Blood samples were taken before, after and 2 h after the two strain cycles and were analysed on histamine by ELISA and on cortisol by LIA (both assays: IBL, Hamburg).

No differences between the four strain types could be obtained in plasma histamine and cortisol, respectively. Histamine levels were between 4.2±0.9 and 10.0±1.9 nmol/l plasma (mean±SEM). Histamine tended to decrease after strain in summer run ($P=0.14$) and to increase 2 h after strain in summer search ($P=0.11$). Cortisol levels were between 28.4±2.4 and 56.1±17.9 nmol/l plasma (mean±SEM). However, cortisol decreased significantly after search strain in winter and increased ($P<0.05$) thereafter (2 h after strain) again on basis level. Plasma histamine only tended to correlate with cortisol level after strain in summer run ($R=0.50$; $P=0.10$).

None of the four training strains led to stress in the rescue dogs. Furthermore, histamine levels were not elevated, even if outdoor temperature was partly extreme. The diminished cortisol level after search strain in winter could not be clearly explained. The expected relationship between cortisol and histamine levels was not observed.

Single-cell RT-PCR analysis of adenosine deaminase expression in rat histaminergic neurons

Amberger B.T., Sergeeva O.A. and Haas H.L.

Department of Neurophysiology, Heinrich-Heine-University, Düsseldorf, Germany

TM neurons in the posterior hypothalamus can be selectively stained by an immunoreaction directed against the total pool of adenosine deaminases isolated from bovine spleen (Staines et al. 1987). The functional meaning of this expression and the subtypes of the enzyme at this location are unknown. We developed protocols for either competitive (followed by the restriction analysis) or subtype-specific amplification of 3 known adenosine deaminases acting on RNA (ADAR). The mRNA encoding for ADAR1 was most frequently detected (in 91% of histidine decarboxylase (HDC)-positive neurons) followed by ADAR2 (79%) and ADA3 (33%). In 38 % of the cells ADAR1 represented either the major or the only deaminase type. In order to estimate the functional impact of these enzymes we determined the editing status of the serotonin 2C receptor, where the sites A and B were found to be edited by ADAR1 while the sites C and D were edited by ADAR2. Site A was edited in all neurons, site B in 90 % of the neurons, among those 2 cells lacked ADAR1. In 3 cells where site B was not edited ADAR1 was expressed. There was a negative correlation in the editing of C and D sites. As we did not find a significant correlation between editing and ADAR expression we conclude that ADAR must fulfill a yet unknown function besides RNA editing. Editing may be a stochastic, constitutive process demanding additional factors in native cells.

Effects of genistein on rat ileum smooth muscle contraction

B.Y.C. Wan[a], K.H. Peh[a], F.L. Pearce[a] and E.S.K. Assem[b]

Departments of Chemistry[a] and Pharmacology[b], University College London, [a]20 Gordon Street, London WC1H 0AJ, [b]Gower Street, London WC1E 6BT, UK

Tyrosine kinase modulates muscle contractility [1] and histamine release [2]. We have studied (a) the effects of genistein (Gen, a tyrosine kinase inhibitor) on rat ileum muscle contraction (IMC) induced by NaF (a non-specific G-protein activator) and carbachol (Car, a muscarinic receptor agonist); (b) whether the action of Gen would be affected by disodium cromoglycate (DSCG, "mast cell stabilizer"), aminoguanidine (AMG, nitric oxide synthesis inhibitor), sodium nitroprusside (SNP, nitric oxide donor), N-tosyl-L-phenylalanine chloromethyl ketone (TPCK, α-chymotrypsin inhibitor), as well as calcium ionophore A23187.

IMC was studied as previously described [3]. NaF and carbacol at stimulatory concentrations were added to one ileum preparation, once before and once after the 15 min incubation with a given concentration of Gen. AMG, DSCG, SNP and TPCK were administered together with Gen for 15 min, whereas A23187 was given 15 min before Gen. The results were expressed as the percentage reduction of stimulant-induced contraction.

Sub-maximal concentrations of NaF (10^{-2} M) and carbachol ($5x10^{-7}$ M) induced a >50% contraction above the basal level. Gen (10^{-7} – 10^{-5} M) produced dose-related relaxation of NaF-induced IMC ($IC_{50} = 10^{-6}$ M). Gen at the same concentrations was less effective against Car-induced IMC ($IC_{50} = 10^{-4}$ M). DSCG (10^{-4} M) markedly and significantly ($p<0.01$) reduced the relaxant effect of Gen (10^{-6} M) on NaF-induced IMC, from a percentage reduction of 48.3±4.6 (Gen+NaF) to 12.1±4.3 (DSCG+Gen+NaF). A23187, AMG, SNP and TPCK (all at 10^{-4} M) had moderate inhibitory effects on the action of Gen on NaF-induced IMC. A23187, DSCG, AMG, SNP as well as TPCK had little effect on the action of Gen on Car-induced IMC. DSCG had little effect on NaF stimulation.

In addition to its membrane stabilizing effect, DSCG is an inhibitor of stimulated G-protein activity [4]. It is possible that DSCG inhibits the relaxant effect of Gen on NaF-induced IMC via a G-protein coupled signal transduction pathway.

[1] Hollenberg MD. TIPS 1994; 15: 108.
[2] Kawakami T, *et al.*, J Immunol 1992; 148: 3513.
[3] Wan BYC, Peh KH, Yue SC, Assem ESK and Pearce FL. Inflamm Res, *Supplememt 1* 2002; 51: S15.
[4] Klinker JF and Seifert R. Inflamm Res 1997; 46: 46.

Börje Uvnäs-Symposium

Effect of cyclosporin-A on secretion from intact and permeabilised mast cells

Harriet Broyd[a], Anna Koffer[a], El-Sayed K Assem[b]

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The effect of cyclosporin A, CSA, an inhibitor of calcineurin, a calcium/calmodulin-dependent phosphatase, on secretion from intact and permeabilised mast cells was examined. Two types of mast cells were used: primary rat peritoneal mast cells (RPMC) purified from peritoneal lavage, and a human mast cell line (HMC-1).

Previous results have shown that intact RPMC and RBL-2H3 (rat basophilic leukemia cell line) cells are affected differentially, according to the calcium dependence of the secretagogoue. While the responses to the antigen and concanavalin A were highly sensitive to CSA (> 80 % inhibition at 200 nM CSA), those to the basic secretagogues, (e.g. compound 48/80) and to ionomycin were far less sensitive (~20 % inhibition at 200 nM CSA) [1,2]. Here, we found that intact HMC-1 cells, responding to 40 nM PMA or 10 μM ionomycin, were also inhibited by ~20 % by 200 nM CSA.

Permeabilised cell system offers the opportunity to by-pass the calcium dependence (since Ca^{2+} concentrations are strictly buffered) and allows investigations of the final steps of exocytosis. Depletion of calmodulin from permeabilised RPMC does not affect secretion, although it interferes with the associated actin reorganisation [3]. Since calcineurin is a target for calmodulin, it was expected that CSA will have no effect on secretion. Indeed, we have found this to be the case: no effects on either calcium-dependent or calcium independent secretion were observed. Thus, CSA, up to 2 μM, did not inhibit secretion from permeabilised RPMC or HMC-1, responding to EGTA + GTPγS + ATP, to 10 μM Ca^{2+} + ATP or to 10 μM Ca^{2+} + GTPγS + ATP. The results indicate that calcineurin does not participate in the final steps of exocytosis but may be involved in the upstream regulation of calcium influx into the cells, possibly by regulating calcium channels phosphorylation [4].

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Homo-oligomersation of the human histamine H_1 receptor

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G-protein coupled receptors (GPCRs) represent the largest family of receptors involved in transmembrane signalling. Although these receptors were generally believed to be monomeric entities, accumulating evidence supports the presence of GPCRs in multimeric forms. Here, using immunoprecipitation as well as time-resolved fluorescence resonance energy transfer (tr-FRET) to assess protein-protein interactions in living cells, we unambiguously demonstrate the occurrence of dimerisation of the human histamine H_1 receptor. We also show the presence of domain swapped H_1 receptor dimers in which there is the reciprocal exchange of TM domains 6 and 7 between the receptors present in the dimer. Mutation of aspartate^{107} in transmembrane domain (TM) 3 or phenylalanine^{432} in TM6 to alanine results in two radioligand-binding deficient mutant H_1 receptors. Co-expression of H_1 D^{107}A and H_1 F^{432}A, however, results in a reconstituted radioligand binding site that exhibits a pharmacological profile that corresponds to the wild-type H_1 receptor. Interestingly, the H_1 receptor radioligands [^{3}H]mepyramine and [^{3}H]-(-)-*trans*-H_2-PAT show differential saturation binding values (B_{max}) for wild-type H_1 receptors, but not for the radioligand binding site that is formed upon co-expression of H_1 D^{107}A and H_1 F^{432}A receptors, suggesting the presence of different H_1 receptor populations.

Homo-oligomerization of the human histamine H_4 receptor

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Over the last years a growing body of evidence has emerged supporting the concept that G protein-coupled receptors (GPCRs) can form oligomers in living cells. In contrast to early indirect evidence this evidence is more convincing due to the use of biophysical methods, such as forms of resonance energy transfer (RET). Using fluorescence resonance energy transfer (FRET) oligomerization of e.g. the somatostatin receptor [1] and thyrotropin receptor [2] has been shown. Another method to determine receptor dimerization that uses RET is bioluminescence resonance energy transfer (BRET). The use of bioluminescence is advantageous over fluorescence in that it does not cause photobleaching of the donor and premature excitation of the acceptor. This method has already been successfully applied to determine oligomerization of e.g. the β adrenergic receptor [3] and the CXCR4 chemokine receptor [4]. A disadvantage of FRET and BRET is that it is not possible to define the cellular location of the signal. Two variations of FRET namely time resolved FRET (trFRET) and photobleaching FRET have been useful in overcoming this problem. In the case of trFRET a small epitope tag is fused to the N-terminus of the receptor. Antibodies fused to europium cryptate or allophycocyanin targeted at these tags than function as donor or acceptor. Since the tag is extracellular the antibodies can only bind to receptors that have been correctly inserted in the membrane.

Oligomerization has already been shown to occur for the histamine receptors as well. Using trFRET homo-oligomerization of the histamine H_1 receptor has been shown [5, 6]. Using immunoblotting it has also been suggested already that the histamine H_2 receptor form homo-oligomers [7]. By using both trFRET and BRET we report here that also the histamine H_4 receptor can form homo-oligomers and that these oligomers are present at the cell surface.

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Improvement in spatial memory by amthamine, a histamine H2-receptor agonist, requires hippocampal ERK activation

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We reported that stimulation of H2 receptors activated extracellular signal-related kinase (ERK) in hippocampus, and improved fear memory [1]. ERK-mediated cellular events in the hippocampus are critical for spatial memory [2]. Therefore, we investigated the effects of intra-hippocampal administration of amthamine, an H2 receptor agonist, and of the MEK inhibitor U0126 on the Morris water-maze performed by male rats (Long-Evans, 280-330 g). The maze consisted of a circular pool filled with opalescent water at 24°C. Animals learned to find the escape, submerged platform, that was kept in a constant position. Escape latencies were measured on day 1, 2 and 4, and a 10-trial/day protocol was used. Immediately after completion of second-day trials, rats were anesthetized and received bilateral intra-hippocampal infusions of amthamine (0.16 ng, n=9 or 1.6 ng, n=15), U0126 (2 µg, n=10) or a solution containing U0126 and amthamine (2 µg and 1.6 ng, respectively, n=9). Controls received bilateral injections of saline (n=10). Rats treated with 1.6 ng amthamine spent significantly less time in locating the escape platform on day 4 than on day 2 (P=0.019, paired Student's t test). All other groups did not show significant differences. It should be noted that blockade of ERK activation by co-administration of U0126 completely prevented the time decrease elicited by amthamine. Although the hippocampus receives only a moderate histaminergic innervation, histamine exerts many effects in this region. These findings suggest that H2 receptor activation facilitates spatial memory with a mechanism involving ERK production, thus providing major insight into histamine regulation of hippocampal function and the mechanisms underlying learning.

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Modulation of Akt/GSK3-β axis as new signaling properties of the histamine H_3 receptor

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The human and rat histamine H3 receptor (H3R) show an agonist-induced inhibition of adenylate cyclase through Gi/o G-proteins (Lovenberg et al., 1999, Lovenberg et al. 2000). Additionally, Gi/o-mediated activation of phospholipase A2 as measured by the arachidonic acid release (Morisset et al., 2000) and MAPK phosphorylation upon activation of the histamine H3 receptor has been shown (Drutel et al. 2001).

Here we report the Gi/o mediated H3R signaling via the Akt/Glycogen synthase 3 (GSK3) axis in SK-N-MC cells stably transfected with the H3R. Immepip, a H3R agonist, induced activation by phosphorylation of Ser473 on Akt, reaching maximal at 10 min. The activation of Akt can be inhibited by the H3R antagonist thioperamide and by wortmannin and PTX, but not by U0126, a specific MEK inhibitor, suggesting activation occurring via a Gi/o-mediated activation of PI-3K. Subsequently, immepip also caused deactivation of GSK3-β by inducing phosphorylation at Ser9, reaching maximal at 5-10 min.

The serine/threonine kinase Akt is known to have a critical role in neuronal development and function. GSK3, a downstream effector of Akt, has a prominent role in neurodegenerative disease. In vitro and in vivo evidence points to a key role for GSK3 in promoting neurodegeneration and in Alzheimer's disease plaque and neurofibrillary tangle formation.

There is experimental evidence that drugs targeted at H3R could be beneficial for neurodegenerative diseases such as Alzheimer and Parkinson's disease. Identification of this newly discovered signaling property of the H3R could add new understanding of the role of histamine and the H3R in brain function and pathology.

Ligand-Directed Multifunctional Signaling of Histamine H_1 Receptors

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Histamine activates the H_1 GPCR to stimulate both the phospholipase (PL) C/inositol phosphates (IP) and the adenylyl cyclase (AC)/cAMP intracellular signaling pathways in a variety of cell/tissue preparations and transfected clonal cell lines. Two H_1 ligands, developed in our laboratory, (–)-*trans*-1-phenyl-3-dimethylamino-1,2,3,4-tetrahydronaphthalene (*trans*-PAT) and (±)-c*is*-5-phenyl-7-(dimethylamino)-5,6,7,8-tetrahydro-9*H*-benzocycloheptene (*cis*-PAB), activate H_1 receptors to selectively stimulate AC/cAMP formation and PLC/ IP formation, respectively, in CHO-H_1 cells. *Trans*-PAT and *cis*-PAB also are functionally selective antagonists of H_1-linked AC/cAMP and PLC/IP signaling, respectively. The molecular structural determinants for *trans*-PAT vs. *cis*-PAB differential binding to the H_1 active site that leads to differential activation of H_1 signaling pathways likely is due to stereochemical factors and steric influences of the dimethylamino and pendant phenyl moieties. Results suggest ligands can be designed to selectively activate or block different intracellular signaling pathways for the same GPCR. Since most untoward cardiovascular, respiratory, and gastrointestinal H_1 receptor-mediated effects proceed via the PLC/IP pathway, PAT/PAB-type agonists that selectively enhance H_1-mediated AC/cAMP signaling provide a mechanistic basis for exploiting H_1 receptor activation for drug design purposes.
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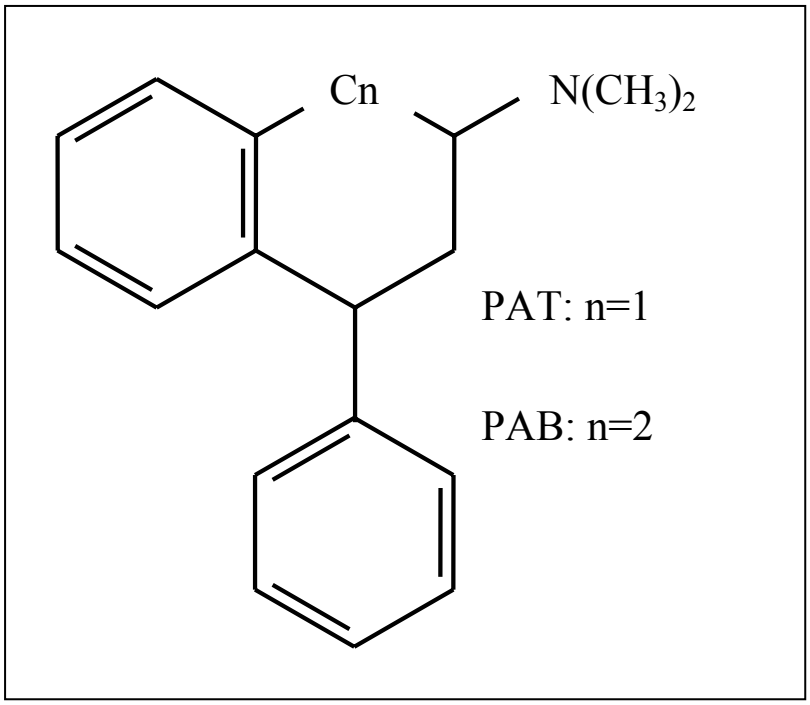

Histamine delays STAT6 phosphorylation in atopic human lymphocyte cell cultures

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Recent studies have shown that histamine influences the phosphoregulation of the signal transducer and activator of transcription (STAT6). In the present study we investigated the differences in the downstream signalling in lymphocytes from non-atopic and atopic patients ex vivo. PBMC from atopics (adult, IgE > 1000 IU) as well as sex and age matched non-atopics (IgE < 100 IU) were stimulated with phytohemagglutinin (PHA) and incubated for 3 days. IL-4 and IFNgamma were measured by ELISAs. Histamine and thioperamide were added alone or in combination 4 hours post-plating. The MTT-test was used to examine cell proliferation. Western blots were performed for detection of latent STAT6 and phosphorylated STAT6 (aSTAT6). During PHA-stimulation of lymphocyte cell culture for 3 days, the expression of latent STAT6 was increased in the atopic compared to the non-atopic derived samples. However, aSTAT6 was maximal 7 hours post-plating and was completely decreased at the end of the 3 day culture period in both atopics and non-atopics. Addition of bolus histamine (10-8M and 2 x 10-6M) did not cause any response in non-atopics but concentration related increasing response phosphorylation of aSTAT6 in the atopic cells. Thioperamide inhibited the augmentation of phosphorylation alone ($p < 0.001$, $n = 6$; Student t) and in combination with histamine ($p < 0.05$, $n = 6$; Student t). Future work must clarify the function of histamine receptor isotype and its particular role in STAT6 phosphorylation in atopy.

Tyramine and benzylamine but not histamine partially mimic the adipogenic effect of insulin in a human preadipocyte cell strain

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Histamine may interact with adipose tissue development since mice deficient in histamine by invalidation of histine decarboxylase gene exhibit increased adiposity and leptin levels (Fülöp et al, 2003), and chronic treatments with an H3-antagonist lower body weight gain in obese mice (Hancock, 2003). The decrease in food consumption provocked by H3 antagonists seems to be involved in their mechanism of action that may provide a novel anti-obesity approach. However, to our knowledge, the putative direct influence of histamine on adipocyte differentiation has never been studied in man. Since we recently reported that histamine was unable to directly promote adipogenesis in murine preadipocyte lineages (Subra et al, 2004), we tested the influence of histamine in a human preadipocyte cell strain with high capacity for adipose differentiation (Wabitsch et al, 2001). This cell strain, derived from an adipose depot of an infant with Simpson-Golabi-Behmel syndrome (SGBS) was cultured in a DMEM F12 medium containing insulin, T3, cortisol and transferrin. Cells accumulated lipids after a 4-day exposure to 25 nM dexamethasone, 200 µM IBMX and 2 µM rosiglitazone. Ten days after confluence, this treatment resulted, in an accumulation of 27-36 µg triacylglycerol/well (TG), while cells at confluence contained 2 µg TG/well. The replacement of 20 nM insulin by 1 mM histamine during 10 days did not modify cell number and allowed to observe only 19 ± 1 % of the insulin-promoted lipid accumulation (n = 5 passages). Under the same conditions, 1 mM tyramine or benzylamine provocked a TG accumulation that reached 37 ± 3 and 40 ± 5 % of the insulin effect, respectively. This was in agreement with the appearence of the mRNAs corresponding to the genes responsibles for the SSAO activity (semicarbazide-sensitive amine oxidase) involved in the adipogenic effects of amines in murine preadipocytes. Therefore, as already observed in mature adipocytes, histamine is poorly oxidized by human preadipocytes and does not behave as other amine oxidase substrates that exhibit direct insulin-like adipogenic effects. However, these observations do not exclude that histamine may indirectly influence fat cell recruitment.
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A study on the mast cell stabilising properties of *Sargentodoxa cuneata*

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Sargentodoxa cuneata is an important herbal medicinal remedy used in traditional Chinese medicine. The stem has antibacterial, carminative, antirheumatic, diuretic and antihelmintic properties [1]. A decoction or tincture has also been used to treat appendicitis, abdominal pain, metrorrhagia, amenorrhea, dysmenorrhea, rheumatoid arthritis and traumatic injuries [2]. Recent studies have suggested that crude extracts or isolated constituents of this herbal drug display anti-inflammatory properties [2]. We investigated the ability of methanol and DCM extracts of *S. cuneata* to inhibit the release of histamine from rat peritoneal mast cells, using the method described by Kim et al [3] was investigated. Methanol extracts of *S. cuneata* (2mg/ml) inhibited histamine release induced by compound 48/80 and calcium ionophore A23187. The respective inhibition values were 86.61±10.78% and 36.87±8.03%. The DCM extracts (2mg/ml) of *S. cuneata* totally inhibited histamine release induced by compound 48/80. Further work is being carried out to isolate and characterize the principle mast cell stabilising constituents.

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Hiroshi Wada Memorial symposium

The pathogenic role of histamine in Alzheimer disease

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Histamine (HA) is a pleiotropic monoamine with multiple functions in the CNS. Major roles of HA in brain function are region- and receptor-specific. Age-related changes in HA levels and HA receptors in the CNS also follow an area-specific pattern, with an antagonic profile in areas of synthesis and areas of release. Both neuronal HA and non-neuronal HA pools (e.g., mast cell-, glia-, endothelial-related HA) are important contributing factors in the pathogenesis of several CNS disorders. Growing evidence indicates that neuroinflammatory reactions accelerate neuronal death in Alzheimer disease (AD). Brain HA interacts with several inflammatory cytokines in the CNS where complex HA-cytokine networks regulate astrogliosis, microglia activation and neuroinflammatory phenomena. High levels of HA have been documented in postmortem brain tissue, CSF, and blood of patients with AD. In addition, peripheral HA levels are modified in parallel with different pathogenic and genomic events along the clinical course of AD, emerging as a potential biological marker for AD under different conditions. The main conclusions from different studies carried out in our institution during the past five years are the following: (a) blood HA levels show an age-dependent pattern, progressively increasing with age in AD; (b) changes in HA levels do not correlate with cognitive function; (c) age-related gender differences in blood HA levels are seen in AD patients, with unchanged age-related values in females and increasing HA levels in males; (d) blood HA levels are markedly decreased in APOE-4(+) carriers who do not show any age-related variation whereas APOE-4(-) carriers exhibit an age-related increase; (e) the lowest levels of blood HA are present in patients with the APOE-4/4 genotype; (f) the three major APOE genotypes (3/3, 3/4, 4/4) show a differential age-related profile in blood HA levels; (g) bigenic haplotypes integrating the allelic variants of the APOE and PS1 genes reveal a clear genotype-dependent HA profile indicating that HA levels are associated with genotypes of high risk for AD, and the lowest levels of HA appear in the 3412, 3422, 4411 and 4412 haplotypes; (h) bigenic haplotypes also show a differential age-related variation in blood HA levels; (i) HA levels are not influenced by PS1 genotypes, and age- and PS1 genotype-related changes are practically identical; (j) serum ACE activity does not influence HA levels and vice-versa; (k) little changes are seen in the levels of HA associated with ACE genotypes; (l) lymphocyte marker variation does not influence HA levels; (m) changes in blood HA do not affect beta-amyloid protein, ApoE or nitric oxide levels; (n) increased levels of HA may contribute to increase moderately brain blood flow velocity and to significantly increase the resistance index in the territories irrigated by the arteries of the circle of Willis; (o) brain HA may not be relevant for memory function, but neuronal HA is involved in important functions of the CNS, such as neuroendocrine regulation, appetite, sleep-wakefulness cycle, circadian rythms, arousal, endothelial function, and neuron-glial networks; (p) the phenotypic profile of blood HA in AD seems to be genetically regulated; and (q) cerebrovascular alterations seem to be associated with changes in HA levels reflecting a potential role of HA in cerebrovascular dysregulation and neuroinflammatory reactions in AD. All these data together suggest that dual mechanisms (toxic vs trophic) associated with HA might be involved in the complex pathogenic cascade of deleterious events that influence apoptosis and neuroinflammation in AD.
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(1-[4-(3-Piperidin-1-ylpropoxy)benzyl]piperidine): A Template for the Design of Potent and Selective Non-Imidazole Histamine H3 Receptor Antagonists.

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(1-[4-(3-Piperidin-1-ylpropoxy)benzyl]piperidine): A Template for the Design of Potent and Selective Non-Imidazole Histamine H3 Receptor Antagonists. Nicholas I Carruthers Johnson and Johnson Pharmaceutical Research and Development, L.L.C. 3210 Merryfield Row, San Diego, CA 92121, USA Subsequent to the discovery and cloning of the human histamine H3 receptor, and the recognition that it is expressed predominantly in the central nervous system [1], we initiated a screening program to identify novel ligands. Our objective of a small molecule, non-imidazole, with good brain penetration was successful, affording a range of related diamine-based ligands [2, 3]. Analysis of the structural features of these ligands, and those reported by several other groups [4, 5, 6], resulted in the generation of an elementary bis-piperidine containing pharmacophore (1-[4-(3-piperidin-1-ylpropoxy)benzyl]piperidine) [7]. With a potent and selective compound available the pharmacological effects of H3 receptor blockade on arousal, cognition and food intake were investigated. These initial in vitro and in vivo pharmacological observations will be highlighted together with our successful modification of the bis-piperidine template to design molecules with desirable PK/PD and ADME properties.

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Cannabinoids modulate the activity of histaminergic neurons

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Activation of cannabinoid receptors in the CNS induces an array of behavioural effects including alteration of cognition, locomotion and pain perception [1]. Endocannabinoids modulate information flow in neuronal networks associated with memory information [1], whereas the histaminergic system provides one of the major excitatory source of cortical activation and has direct effects on cognitive processes [2]. We are investigating the interactions between the cannabinoid and histaminergic systems in freely moving rats using the dual probe microdialysis technique: a selective CB1 receptor agonist (ACEA) was infused in the tuberomammillary nucleus (TMN) and histamine contents were determined both in the ipsilateral nucleus basalis magnocellularis (NBM) and the TMN. In the NBM activation of H_1 receptors modulate ACh spontaneous release in the frontal cortex [3] and ameliorates memory in the object recognition test [4]. We first assessed the effect of the H_3 receptor antagonist/inverse agonist thioperamide. Administration of thioperamide (300 nM) in the TMN increased HA release in the NBM by approximately 125% (spontaneous release=0.02±0.01 pmol/15 min; flow rate, 2.0 µl/min) and from the TMN by about 200% (spontaneous release=0.037±0.01 pmol/15 min; flow rate, 2.0 µl/min; (n=3). When ACEA (150 nM) was infused in the TMN for 60 min, HA release increased about 600% in the NBM (spontaneous release=0.03±0.015 pmol/15 min) and approximately 800% in the TMN (spontaneous release=0.55±0.015 pmol/15 min; n=4). 15nM ACEA did not modify HA spontaneous release significantly in either the TMN or the NBM (n=2). This is the first report of cannabinoids effect on histamine release in the CNS.

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Comparison of the receptor occupancy kinetics of levocetirizine at peripheral and central histamine H_1 receptors after oral administration in the guinea pig.

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The main side effects of the first generation antihistamines are sedation, drowsiness and impaired performance due to their action on histamine H_1 receptors in the brain. The second generation antihistamines (see [1] for review) are characterised by reduced brain penetration as demonstrated [2, 3] by positron emission tomography investigations in adult volunteers. An occupation of approximately 30 % of histamine H_1 receptors in the human cerebral cortex [4] seems to be tolerated before emergence of the first side effects.
The present experiments were designed to determine histamine H_1 receptor occupation kinetics at peripheral and central sites in guinea pig in order to estimate if such a model could be predictive for estimating potential sedative effects in man. Two doses of levocetirizine (0.1 or 1 mg/kg corresponding approximately to 1 and 10 fold the therapeutic dose) were orally administered to guinea pigs 1, 2, 4, 8 or 16 hours before determining, in the same animal the effect on histamine contractions of the isolated ileum (peripheral effect) or on the ex-vivo binding to histamine H_1 receptors in the cerebral cortex (central effect). Solvent (0.9 % NaCl) or chlorpheniramine (2 mg/kg) were used to define 0 and 100 % ligand displacement respectively.

At the dose of 0.1 mg/kg, no occupancy of cortex histamine H_1 receptors was observed whereas 50-70 % of the receptors were occupied at the 1.0 mg/kg dose. Receptor occupation was maximal between 2 and 8 hours and began to wane 8 hours after the injection of levocetirizine. A 4 and 33-fold shift to the right, without any decrease of the maximal amplitude of the concentration-response curve to histamine was observed on the ileum in animals treated with 0.1 and 1.0 mg/kg levocetirizine respectively. These findings are comparable to results obtained with direct in vitro testing of levocetirizine in the tissue bath [See 5]. Effects were maximal after 1-2 hours and began to disappear 4-8 hours after injection of levocetirizine. However, a 2 fold shift in the histamine concentration-response curve was still observed 16 hours after treatment with 1 mg/kg.

The present results demonstrate that it is possible to clearly separate the peripheral (therapeutic effects) from the central (side effects) antihistaminic effects of levocetirizine. A dose of levocetirizine (0.1 mg/kg) producing very effective blockade of histamine H_1 receptors in the periphery had no effect on such receptors in the cerebral cortex. Higher doses of levocetirizine (1 mg/kg) were necessary to produce an effect at central sites and even with doses many times the peripherally active dose, this remained only partial.

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Achievement of Behavioral Efficacy and Improved Potency in New Heterocyclic Analogs of Benzofuran H3 Antagonists

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The CNS histamine system plays an important role in regulating brain physiology and function through H1, H2, H3 receptors, and indirectly, through other neurotransmitter receptors [1]. H3 antagonists induce the release of histamine and other neurotransmitters, and compounds from a variety of chemical series have shown efficacy in animal models [2]. There is intense interest in finding candidate compounds with higher potency and better efficacy profiles [3], in order to justify subsequent clinical trials. The benzofuran-based H3 antagonist ABT-239 has shown nanomolar potency at H3 receptors and efficacy in rodent models of cognition and attention at doses of 0.01 - 0.1 mg/kg [4, 5]. By using microdialysis, we now find that in rats, both ABT-239 and ciproxifan induce elevations of acetylcholine in prefrontal cortex and in hippocampus at the same doses that enhance cognition and attention animal models. Many other analogs of ABT-239 have now been investigated to determine the relationship between structure, receptor binding potency, and efficacy in behavioral models. In several series, potent behavioral efficacy is found in new analogs that replace the 4-cyanophenyl moiety of ABT-239 with (1) aryl ketones, (2) substituted aryls, and (3) specific heterocycles. In the most active compounds, H3 binding potency was found to be as low as 100 pM at human and rat H3 receptors, and it was confirmed that the compounds act as inverse agonists (GTP-γ-S binding). The synthesis and SAR of these compounds in binding and functional assays will be described, along with some behavioral, pharmacokinetic, and other important properties.

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Histamine release from blood cells and serum ECP in patients with asthma, during and after a mild pollen season

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Activated mastcells and eosinophils are frequently found in bronchial mucosa and in circulating blood of asthmatics. These cells may release histamine and eosinophil cationic protein (ECP) when triggered by certain stimuli. Levels of ECP in serum have been used to monitor fluctuations in clinical asthma. The aim of this study was to evaluate whether histamine release from circulating mixed blood cells and/or ECP in serum would respond to seasonal changes in airborne birch pollen. The diagnostic capacities of these tests were also to be compared. Twenty-two patients with mild asthma participated in this study. All patients exhibited merely mild symptoms during pollen season. Lung function tests as well as collection of blood were done during and four months after a mild pollen season. Twelve of 22 asthmatics were under treatment with inhaled corticosteroids. A commercially available ELISA immunoassay (IBL, Germany) was used for histamine assays and ECP was measured by radioimmunoassay (Pharmacia Diagnostica, Sweden). Nonparametric statistical tests and analyses of receiver operated curves (ROC) were done (Medcalc Statistical Software, Belgium). Median values (lower – upper quartiles) are given. Significantly lower values of IgE stimulated histamine release from circulating blood cells were recorded after- relative to during ongoing pollen season (25.4 [16.5-53.1] ng/mL vs.40.4 [21.2-73.8] ng/mL, p=0.03). Also serum ECP levels (5.9 [4.5-6.6] microg/L vs. 13.5 [9,2-20] microg/L, p=0.002) and airway deadspace (172[142-196] mL vs. 179 [159-246] mL, p=0.02) were lower four months after season. The capacity to correctly diagnose a change in airway deadspace was evaluated by means of ROC analysis and the sensitivity of histamine release (89%) and serum ECP (80%) were found to be of similar magnitude. Specificity of histamine release tended to be lower (33%) than that of serum ECP (56%). Analysis of areas under the ROC curves however, revealed no statistically significant difference between the two tests.

We conclude that the release of histamine from mixed blood cells and serum ECP levels had similar capacity to correctly diagnose minute changes in airway deadspace, resulting from airborne birch pollen exposure in asthmatics.

The influence of serotonine, the 5-HT_2 and 5-HT_3 receptor agonists on the haemodynamics of the isolated, constant pressure perfused, rat heart

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In our previous study (Inflamm Res 48, Supp. I (1999) S96-S97) we showed the cardioprotective action of serotonine and the 5-HT_3 receptor agonist. The aim of the present study was to determine the influence of the intracoronary infusion of 5-HT_2 (alfa-methyl-5-hydroxytryptamine) and 5-HT_3 (m-chlorophenylbiguanide) agonists on the heart rate, coronary flow and contractile force of the isolated, constant pressure perfused rat heart.

The Wistar male rats (randomly divided into 3 groups, 6 animals each) were sacrificed after intraperitoneal administration of heparin and pentobarbital anesthesia. Hearts were excised and rinsed in ice-cold Krebs buffer until arrest. They were mounted on the Langendorff apparatus (Radnoti, USA) and perfused with standard Krebs solution saturated with the mixture of 0_2 (95%) and CO_2 (5%). They were allowed 30 minutes for stabilization and afterwards exposed to intracoronary infusion of 10^{-3}mol/l solution of alfa-methyl-5-hydroxytryptamine, m-chlorophenyl or 5-hydroxytryptamine. The coronary flow, heart rate and the contractile force curve morphology were recorded (PowerLab) for further evaluation. After the experiment the hearts were dried and weighted in order to determine the coronary flow/heart mass ratio. The results were expressed as means±SD and statistical significance was determined according to ANOVA.

Neither the mean mass of hearts (0,179g±0,027; 0,210g±0,027 and 0,199g±0,012 respectively), nor the flow/mass ratio (37,857ml/min/g±4,926; 35,158ml/min/g±3,537; and 38,879ml/min/g±7,428 respectively) did not significantly differ between groups. The heart rate increased by 17% in serotonine group, decreased by 7% in 5-HT_2 receptor agonist group and increased by 25% in 5-HT_3 receptor agonist group, however the infusion of serotonine and its selective agonists did not significantly affect neither the amplitude nor width of the contractile force curve tracing.

Thus we may conclude that the infusion of serotonine and selective 5-HT_3 receptor agonist has a positive chronotropic effect, but none of the compounds has an inotropic effect in the described setting of experiment.

Reduced open-field exploration, increased anxiety and improved rotarod performance concomitant with alterations in brain acetylcholine and monoamine Systems after Histidine-Decarboxylase gene knockout in 129/Sv mice

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Histamine has been implicated in exploratory behaviors, emotionality, reinforcement and memory. However, due to the low selectivity of the pharmacological tools and lesion techniques used no clear-cut picture regarding the behavioral functions of brain histamine has emerged. In the present study we investigated some behavioural and neurochemical effects of mice deficient for the HDC gene which are unable to synthesize histamine from its precursor histidine. The HDC knockout mice showed reduced exploratory activity in a small and even more pronounced in a big-sized open-field, as well as in an 8-arm maze. Whereas repeated H_1 receptor antagonist pyrilamine treatments dose-dependently reduced rearings in wild type mice, HDC-/- did not respond to pyrilamine. In the rotarod-test HDC-/- mice performed superior to wild type controls. In two separate measures of unconditioned anxiety HDC-/- mice behaved more anxious than controls. Neurochemical assessments revealed that the HDC-/- mice had significantly higher acetylcholine concentrations in the frontal cortex and neostriatum. Furthermore, the HDC-/- mice had higher DOPAC concentrations in the neostriatum and cerebellum, higher DOPAC/DA ratios in the neostriatum and ventral striatum and a significantly higher 5-HIAA/5-HT ratio in the frontal cortex relative to the wild type mice. These results suggest an important role of brain histamine in motor coordination, exploratory behaviors, emotionality, and brain neurotransmitter concentrations.
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Histamine-evoked cortico-striatal synaptic plasticity and short-term-potentiation are impaired in rats with porto-caval anastomosis

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Among the symptoms of hepatic encephalopathy (HE) are disturbances of motor and cognitive functions involving the basal ganglia. We investigated tetanus-evoked and histamine-mediated synaptic plasticity in the cortico-striatal pathway of rats subjected to portocaval anastomosis (PCA, animal model of HE). Tetanic stimulation in magnesium-free solution evoked short-term potentiation, which was followed by long-term potentiation in the control group (sham operated), both these forms of synaptic plasticity were significantly impaired in PCA rats. Bath-applied histamine (10μM) induced a larger long-term depression (LTD_{hist}) of field potentials in sham-operated animals compared to PCA rats. The LTD_{hist} deficit in PCA rats may be due to the high histamine level in their brains while the impairment of tetanus evoked STP could depend on a functional deficit in NMDA-receptors. Real-time RT-PCR did not reveal any differences in NMDA- and in histamine-receptor (H1, H3) expression, while heme oxygenase-1 and the peripheral-type benzodiazepine receptors were up-regulated in the striatum of PCA rats.

In conclusion, cortico-striatal synaptic plasticity is impaired in the PCA model of mild hepatic encephalopathy.

Börje Uvnäs-Symposium

Mast cell mediator release after endobronchial challenge with AMP in normal subjects

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Background: Adenosine 5'-monophosphate (AMP) causes bronchoconstriction in atopic subjects but has no effect on non-atopic non-asthmatic subjects. Endobronchial AMP challenge has previously been shown to cause mast cell mediator release in asthmatic subjects but it is unknown if a similar response occurs in atopic non-asthmatic and non-atopic non-asthmatic controls who have no response to inhalation AMP challenge. This study examined the change in mast cell derived products after endobronchial saline challenge and AMP challenges in subjects with and without a positive inhalation response to AMP

Methods: Inhalation challenge with AMP challenge was performed in normal (n=8), atopic non-asthmatic (n=6) and atopic asthmatic (n=7) subjects. Levels of mast cell mediators were measured in bronchial wash samples after endobronchial adenosine challenge and after placebo endobronchial saline challenge.

Results: PC_{20} AMP of asthmatic subjects (median{IQR} 3.6{2.3-7.9) mg/ml) was significantly lower when compared with that of either atopic (432.0 {144.8-640}) or normal subjects (none responded to highest tested dose of 640 mg/ml) ($p<0.01$, $p< 0.01$ respectively). None of the mediator concentrations differed significantly between pre-saline and pre-AMP values There were significant increases in histamine, tryptase, protein and PGD_2 ($p = 0.02$, 0.02, 0.01, 0.01 respectively) after AMP challenge compared to saline in non-atopic, non-asthmatic subjects. There was no significant increase in any mediator in either of the other two groups.

Conclusion: This study suggests a dissociation between mediator release and bronchoconstriction in response to AMP.

Orexin / dynorphin neurons control GABAergic inputs to histaminergic neurons

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High activity of the histaminergic neurons in the tuberomamillary nucleus increases wakefulness, and their firing rate is highest during waking and lowest during rapid eye movement sleep. The tuberomamillary neurons receive a prominent innervation from sleep-active GABAergic neurons in the ventrolateral preoptic nucleus, which inhibits them during sleep. They also receive an excitatory input from the orexin and dynorphin containing neurons in the lateral hypothalamus, which are critically involved in sleep regulation and whose dysfunction causes narcolepsy. We have used intracellular recordings and immunohistochemistry to study if orexin neurons exert control over the GABAergic inputs to tuberomamillary neurons in rat hypothalamic slices. Dynorphin suppressed GABAergic inputs and thus disinhibits the tuberomamillary neurons, acting in concert with orexin to increase the excitability of these neurons. In contrast, both orexin-A and orexin-B markedly increased the frequency of GABAergic potentials, while co-application of orexin and dynorphin produced responses similar to dynorphin alone. Thus orexins excite TM neurons directly and by disinhibition, gated by dynorphin. These data might explain some of the neuropathology of narcolepsy.

Use of novel, non-imidazole inverse agonist radioligands to define histamine H_3 receptor pharmacology.

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The binding characteristics of histamine H_3 receptors have been determined using a variety of agonist and antagonist radioligands to date. We have synthesized two potent and selective non-imidazole H_3 receptor antagonist/inverse agonists with cognitive enhancing properties in rodents[1,2], A-317920 (furan-2-carboxylic acid (2-{4-[3-(4-cyclopropanecarbonyl-phenoxy)-propyl]-piperazin-1-yl}-1-methyl-2-oxo-ethyl)-amide) and A-349821 (4-{4-[3-((2*R*,5*R*)-2,5-dimethylpyrrolidinyl)propoxy]phenyl}phenyl-morpholin-4-ylketone), that were radiolabeled for additional H_3 receptor binding characterization. [^{3}H]-A-317920 demonstrates high affinity only for rat brain cortical and cloned H_3 receptors with K_d values of 0.18 and 0.21 nM respectively, whereas [^{3}H]-A-349821 displays high affinity for human and rat cortex as well as recombinant human and rat H_3 receptors with respective K_d values of 0.111, 0.561, 0.094 and 0.303 nM. Competition studies utilizing [^{3}H]-A-317920 and [^{3}H]-A-349821 were performed on a panel of reference agonist and antagonist H_3 selective compounds using native or recombinant H_3 receptor membrane preparations. A rank order of potency was obtained using both [^{3}H]-A-317920 and [^{3}H]-A-349821 that correlated to that for the H_3 receptor agonist radioligand [^{3}H]-N-α-methylhistamine, suggesting labeling of common receptor sites by both the imidazole agonist and non-imidazole inverse agonist radioligands. In addition, correlation plots indicate that H_3 receptor agonists can be discriminated from H_3 receptor antagonists by use of these non-imidazole inverse agonist radioligands that confer higher binding K_i values to H_3 receptor agonists. A-317920 and A-349821 represent novel, non-imidazole inverse agonists that recognize H_3 receptors with high potency and selectivity. [^{3}H]-A-317920 and [^{3}H]-A-349821 serve as useful non-imidazole H_3 receptor inverse agonist radioligands for the characterization of H_3 receptors in saturation and competition assays using membranes expressing native or recombinant H_3 receptors from rat ([^{3}H]-A-317920 and [^{3}H]-A-349821) and human ([^{3}H]-A-349821).

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Lack of Protease Activated Receptor (PAR) expression in purified human basophils.

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Protease activated receptors are members of a new family of G-protein coupled receptors whose activation is induced by proteolytic cleavage of an extracellular N-terminal domain, exposing a tethered ligand. So far, four receptors have been characterised (PAR-1, -2, -3, -4). Physiological activators of PARs are the proteases thrombin (PAR-1, -3, -4), mast cell-derived tryptase (PAR-2) and trypsin (PAR-2 and PAR-4). PARs are selectively expressed on a variety of immune and other cells. Human basophils are thought to play a role in the pathophysiology of allergic diseases such as asthma. Also, the house dust-mite allergens Der p1, Der p3 and Der p9 have been shown to activate PARs. We postulated that PAR activation, in the contexts of allergens or helminth proteases released by tissue-migrating life stages, could lead to the release of histamine, IL-4 and IL-13 by basophils, further polarizing the Th2 bias seen in allergy and helminth infection. We have therefore studied the expression of specific mRNA in highly purified basophils by RT-PCR. All oligonucleotide primers were strictly specific for the respective PARs (as determined by blasting against the human genome) and were intron-spanning (allowing safe discrimination of genomic contaminants). We also performed functional studies using synthetic agonists of PAR-1, PAR-2 and PAR-4 (TRAP-6; H-Ser-Leu-Ile-Gly-Lys-Val-NH2 and H-Gly-Tyr-Pro-Gly-Lys-Phe-OH) in a broad concentration range (0.01-100 μM). Both the functional assays and the RT-PCR expression analysis did not support the existence of the known PARs on basophils. This finding is consistent with previous work showing that human basophils are unresponsive to trypsin and thrombin [Alic A. et al., Inflamm. Res 50 Supplement 2 (2001) S57-S58].

Histamine H2 receptor regulates GRK2 expression by a dual mechanism in U-937 cell line

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We have previously reported the importance of the time-course of the cAMP response evoked by H2 receptors in the induction of U-937 cell differentiation mediated by H2 agonists (*Mol Pharmacol* 1997; **51**:983-90. *Biochem Pharmacol*. 2000; **60**:159-66. *Mol Pharmacol* 2002; **62**:1506-14).

To analyze the relevance of the intensity of the signal on this final cellular response, we stably overexpressed H2 receptors in U-937 cells. In this system we could achieved H2 agonist-mediated cell differentiation due to the higher cAMP production induced by the agonist (*EHRS meeting 2003, Fernandez et al.*). Remarkably, we observed an increase in GRK2 basal levels, probably as a regulatory feedback mechanism, that was not due to cAMP, since its basal levels were similar in the naïve and surrogate systems, as a consequence of PDE induction.

Since it was largely described that ERK1/2 activity regulates GRK2 expression, we decided to study MAPK role in such modulation. As we expected, it was found that in the H2 receptor overexpression system there were higher levels of ERK-P than in U-937 cells. Consistently, agents that activate this MAPK, produced an increase in GRK expression in both cell lines. On the other hand, agents that modulate cAMP levels, such as H2 agonists, db-cAMP, rolipram and forskolin, were able to diminish MAPK activation and GRK expression in both systems.

The whole of these results suggests that GRK expression has a dual regulation involving inhibition by traditional cAMP pathway, and activation by MAPK cascade. The last mechanism could be the explanation of the observed increase in GRK2 levels in the overexpression system. These alternative mechanisms point out the complexity of cell signaling networks, in which GRK2 plays a key role integrating a large array of stimuli in order to allow cell survival.

The ~36kDa isoform of rat L-histidine decarboxylase is capable of dimerizing but is nevertheless deficient in the binding and decarboxylation of substrate

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Recent studies have proposed the existence of a flexible loop domain in rat L-histidine decarboxylase (residues 330-340 of the rat HDC primary protein sequence) [1,2]. Proteolysis at this domain occurs naturally *in vivo* and gives rise to an inactive 36kDa isoform. To better understand the functional consequences of this proteolysis and the molecular basis for this inactivation, we have used both *in vitro* and *in vivo* approaches to study the dimerization and substrate binding properties of this isoform. Cos-7 cells were transiently transfected to co-express HA-tagged HDC1/339HA and FLAG-tagged HDC1/339FL isoforms. Forty-eight hours after transfection the lysates were analyzed for homo-dimerization, and co-immunoprecipitation studies demonstrated that the HA and FLAG tagged versions of the ~36kDa protein were indeed capable of stringent and specific interactions. To study the ability of this isoform to bind and decarboxylate substrate we over-expressed and purified a recombinant rat HDC1/339His isoform from bacteria. Partially purified protein (>60%) was incubated in the presence and absence of substrate analogues α-fluoromethyl histidine and histidine methyl ester. After one hour the recombinant ~36kDa protein was fractionated on semi-denaturing protein gels, but no changes in electrophoretic mobility could be detected. In contrast, an active HDC1/516His isoform that was purified in parallel showed a clear change in electrophoretic mobility indicative of the successful binding of substrate analogues. We conclude that proteolysis at the flexible loop domain of rat HDC destroys substrate interacting properties, but does not disrupt the dimerization of HDC monomers.

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Gastrointestinal Histamine System in experimental ulcerative colitis in rats *

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Histamine and polyamine systems are involved in tissue inflammatory and repair processes. The present study aimed to evaluate the effect of compounds acting on the histamine system in acetic acid-induced ulcerative colitis in Wistar rats. Ulcerative colitis was induced by intrarectal administration of 4% acetic acid for 15 s. Ketotifen (2.5mg/kg i.g), ranitidine (40 mg/kg, i.p.) or thioperamide (2 mg/kg, i.p) were administered daily for 5 consecutive days, the first immediately following the induction of colonic inflammation. Rats were killed 5 days after instillation of acid and macroscopic colonic lesions as well as gastrointestinal histamine system parameters were assessed. Plasma ceruloplasmin (Cp) activity and myeloperoxidase (MPO) activity in large bowel served as inflammatory markers.

Colonic inflammation was associated with increased myeloperoxidase and matched well with increased plasma ceruloplasmin activity. There was a significant decrease in histamine content all along GI tract from 30% in the stomach up to 60% in the large bowel. Of the biogenic amine related enzyme activities tested i.e. diamine oxidase, histamine N-methyltransferase, MAOB and MAO A all except MAOA changed insignificantly. The lesion scores, the changes in histamine concentration, Cp and MPO activities but not in MAO A activity were attenuated by ranitidine and to an even greater extent by thioperamide treatment. The data are compatible with previous findings of reduced histamine [1] and MAO A substrate, serotonin [2], in Crohn's disease and suggest that interference with histamine receptors can modify inflammatory and repair processes.

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Ornithine and Histidine decarboxylases in hypertrophic and hyperplastic mouse kidney.

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Polyamines and histamine are regarded as growth regulatory substances. Their synthesizing enzymes, ornithine and histidine decarboxylase, respectively, are inducible, responding similarly to a number of stimuli and their expression is strictly controlled at different levels. To gain some insight into the regulation of these enzymes; two experimental models, hyperplastic and hypertrophic murine kidney, were used.

Methods and Materials: Swiss mice (2.5-3 months old) were injected with testosterone 125 mg/kg s.c. 5 days prior to killing for the kidney hypertrophy model or with N10-propargylo-5,8-dideazafolate (CB 3717, 200mg/kg i.p.) or folate (250 mg/kg i.p.) 1 day prior to killing for the kidney hyperplasia model. After sacrificing the animals, the kidneys were collected and stored at –70°C until assay.

Ornithine decarboxylase and histidine decarboxylase activities were measured by $^{14}CO2$ trapping method using the relevant ^{14}C carboxy-labelled aminoacid substrates. Histamine was measured by fluorimetry after separation on Cellex P column, histamine N-methyltransferase (HMT) and monoamine oxidase form B (MAOB) activities were assayed with currently used isotopic procedures

The levels of transcripts of histidine decarboxylase and ornithine decarboxylase were evaluated by using a commercially available DNA microarray-GE Array Kit (SuperArray Inc., USA). Hybridized membranes were exposed to Storage Phosphor Screen (Kodak Eastman, Rochester, NY, USA) for 5 days. Densitometrical analysis was performed with Typhoon 8600 Variable Mode Imager and ImageQuant software (Molecular Dynamics, USA).

Results: Under both hypertrophy and hyperplasia of the kidney, there was a prominent stimulation of ornithine decarboxylase expression observed at the level of enzyme activity and ODC transcript. Unlike that of ornithine decarboxylase, the HDC transcript level was not increased under any of the experimental conditions. Histamine concentrations were markedly increased only in the hyperplastic model. This increase was associated with 2 fold higher histidine decarboxylase activity and no change in the histamine methylation pathway enzyme activities: HMT and MAO B.

The data obtained so far suggest a distinct regulation of the two proteins in these test systems.

Selective H_3 Receptor Blockade: Broad Efficacy in Cognition and Schizophrenia Models

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Cognitive deficits are apparent across a range of human neurological disorders that are currently inadequately treated. Pharmacological blockade of central H_3Rs enhances cognition in rodents [1,2,3] and there is growing interest in the potential utility of selective and safe compounds for treating cognitive impairment [4]. ABT-239 (4-(2-{2-[(2R)-2-methylpyrrolidinyl] ethyl}-benzofuran-5-yl)benzonitrile tartrate) is a novel, highly selective, non-imidazole H_3R antagonist with high affinity for both rat (Ki = 1.4 nM) and human (Ki = 0.4 nM) H_3Rs that has demonstrated broad efficacy across a number of preclinical models. ABT-239 (0.1 mg/kg), as well as several related compounds, significantly improved performance in a 5-trial, repeated acquisition, inhibitory avoidance test in rat pups, a 10-150-fold improvement in potency, with similar efficacy, over other antagonists such as ciproxifan, thioperamide, A-304121 and A-317920. Efficacy was maintained following repeated dosing with ABT-239 in this model. ABT-239 was also highly potent and efficacious (0.01-0.3 mg/kg) in a social memory model in adult rats and enhanced acetylcholine release (0.1-3.0 mg/kg) in rat prefrontal cortex and hippocampus, regions known to be important in cognition. ABT-239 also attenuated natural gating deficits in DBA/2 mice using prepulse inhibition of startle (1.0-3.0 mg/kg) and N40 (1.0-10.0 mg/kg) models. Further, ABT-239 attenuated methamphetamine-induced hyperactivity in mice, without inducing or potentiating any adverse effects such as catalepsy. ABT-239 (3 mg/kg) also enhanced dopamine release in rat prefrontal cortex, but not striatum, consistent with an enhanced efficacy: adverse effects profile. ABT-239 lacked any stimulant-like activity on slow wave EEG, spontaneous activity or behavioral sensitization measures. In summary, selective H_3 receptor blockade with compounds such as ABT-239 represents a novel and safe approach for the treatment of human cognitive disorders. Potential clinical efficacy may extend beyond disorders such as ADHD to include AD and schizophrenia.

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Histamine H1 receptor-mediated histamine H1 receptor gene expression

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[Aim]
The level of histamine H1 receptor mRNA was increased in nasal mucosa of allergy model rats after allergy provocation, and the increase was partially blocked by H1 receptor antagonists. Then effect of histamine H1 receptor stimulation on H1 receptor expression level was examined.

[Methods]
Histamine H1 receptor-expressing HeLa cells were used. HeLa cells were conditioned without fetal bovine serum for 24 hours before experiments. H1 receptor mRNA was determined by real time PCR. H1 receptors were determined by [3H]mepyramine binding assay. The H1 receptor promoter was inserted into a luciferase reporter gene for the promoter assay.

[Results]
Increase in the level of histamine H1 receptor mRNA was induced by the stimulation by histamine of HeLa cells. The increase was blocked with H1receptor antagonists. The stability of H1 mRNA was changed neither in histamine-stimulated nor in control cells.

[Discussion]
The H1 receptor promoter was activated by histamine in HeLa cells. Histamine-induced increase in H1 mRNA level was inhibited by a protein kinase C inhibitor. The histamine-induced increase was also suppressed by dexamethasone.

Hiroshi Wada Memorial symposium

Studies of histamine H1 receptor functions at molecular and physiological levels

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Histamine H1 receptor (H1R) down-regulation was induced in homologous and heterologous manners. The homologous one was abolished in the mutant H1R lacking putative phosphorylation sites, suggesting receptor phosphorylation plays an important role. Heterologous one was mediated by α2 adrenergic or m3 muscarinic receptors. α2-Adrenergic receptor-mediated H1R down-regulation was partially blocked by the protein kinase A inhibitor. However, m3 muscarinic receptor-mediated one was unrelated to the receptor phosphorylation.

H1R up-regulation was induced by the stimulation of H1R. This up-regulation was mediated by H1R gene promoter and protein kinase C activations. The H1R antagonist with inverse agonist action lowered the H1R mRNA level. The H1R up-regulation was suppressed to the basal level by dexamethasone, suggesting that the mechanism of the up-regulation is a target of steroid.

H1R up-regulation with increase in H1R mRNA level was induced in nasal mucosa of allergy model rats after allergy provocation. The increase in H1R mRNA level was suppressed partially by H1R antagonists and completely by steroid. HDC activity with increase in HDC mRNA level and histamine level in nasal mucosa was also increased. The increase in HDC mRNA level was completely suppressed by steroid, but without effect by the H1R antagonist.

Demonstration of H1R expression is a key step for H1R-mediated histamine functions. Placenta showed high H1R mRNA level, and H1R expression was also demonstrated by [3H]mepyramine binding assay and immunohistochemistry using an antibody against H1R. H1R was located in syncytiotrophoblast of chrionic villus in the placenta. The HDC immuno-positive cells were also immunostained by an antibody against chorionic gonadotropin.

Tissue specific alteration of glucocorticoid receptor number in histamine free mice.

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Histidine decarboxylase gene targeted (HDC-KO) mice can be regarded as histamine free model system, feeding the animals with histamine poor diet. Recently a reduced hepatic glucocorticoid receptor (GCR) number was found in histamine free CD-1 mice. Our intention was to check the GCR density in a genetically rather homogenous, inbred murine (BALBc) model. The GCR immmunoreactivity was measured in three peripheral blood cell types (monocytes, granulocytes, lymphocytes) and disaggregated cells of three solid, but tender organs (thymus, spleen, liver) by flow cytometry after direct labelling with anti-GCR IgG-FITC. In the hepatic GCR immunoreactivity a statistically significant reduction was detected in the HDC-KO animals, while in all the other cell types practically there was no difference in the GCR density. Keeping the HDC-KO mice on standard laboratory diet, the aforementioned difference disappeared. In the animals on standard diet a decrease of hepatic histamine content was also detected, but on histamine free diet the histamine content of liver was below the detection limit of the used method (overpressure layer chromatography). The plasma corticosterone concentration of histamine free animals was slightly lower, but not statistically different from their wild type littermates. All together these data suggest that the hepatic GCR number alteration may be general feature of histamine free mice, because it was found in CD-1 and BALBc animals, and also it assumed to be tissue specific for liver (at least regarding the investigated tissues). The disturbed endocrine regulation of HDC-KO animals is emphasized by the fact that the plasma corticosterone level is not elevated in HA-free animals, so the receptor number alteration is can not be a consequence of down regulation of receptor number by corticosterone and it is in correlation with local histamine content of liver.

Notes

Comparison of ex vivo and in vivo binding data applied to brain H_1 histamine receptors

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In vivo binding experiments are often designed to assess brain penetration of compounds and to measure their binding affinity to a given receptor. After injection of both the unlabelled compound and the radioligand, the brain or specific brain regions are dissected and the radioactivity associated with the tissue sample is quantified.

Injection of μCi amounts of the radioligand leading to high costs and high risks of radioactive contamination is a limiting factor with such experiments. In the case of *ex vivo* binding, only the unlabelled compound is administered to the animal. After dissection, the tissue of interest is homogenized in a minimal volume of buffer. The radioligand is added and the binding experiment is performed *in vitro*. If the advantages of such a design are obvious, there are some potential drawbacks. The main one is the dissociation of the unlabelled compound from the receptor due to dilution of the original sample. This will lead to an underestimation of the compound binding affinity, which will be even greater for compounds having fast dissociation rates from their receptors. In this study, we compared the binding properties of chlorpheniramine, levocetirizine and dextrocetirizine administered iv to guinea pigs. Binding to brain H_1 histamine receptors was measured in *ex*- and *in-vivo* experiments. These three compounds have dissociation half-lives from human H_1 receptors of <2 min, 142 min and 6 min respectively [1]. The IC_{50}, expressed in mg/kg, obtained *in vivo* and *ex vivo* 60 min after treatment, are respectively 0.01 and 0.03 for chlorpheniramine, 0.8 and 1.0 for levocetirizine and 5.0 and 5.0 for dextrocetirizine. Results obtained for chlorpheniramine are in agreement with published results [2]. Although chlorpheniramine and levocetirizine display similar affinity in vitro for H_1 receptors [1], the dose of chlorpheniramine to occupy brain H_1 receptors is 80 fold less than that of levocetirizine. The high concentrations of levocetirizine needed to occupy 50% of the brain H_1 histamine receptors (1 mg / kg or the equivalent of 70 mg for an average man which represents 14 times the daily recommended dose of 5 mg) are in line with the non sedating properties of this antihistamine compared to the first generation sedating chlorpheniramine. We conclude that, at least in the case of H_1 histamine receptors, *in vivo* binding experiments may advantageously be replaced by less costly and safer *ex vivo* binding experiments.

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Primary structure and functional expression of two histamine-gated ion channels in *Drosophila melanogaster*

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Histamine is a neurotransmitter and important neuroregulatory compound in vertebrates, where it acts via G-protein coupled receptors. Furthermore it is the neurotransmitter of photoreceptors in insects and other arthropods where it directly activates a chloride channel and mediates rapid, inhibitory responses. Two genes for ligand-gated ion channels activated by histamine have been cloned in *Drosophila* by a functional genomics approach. By systematic expression screening of novel ligand gated anion channel types predicted by a bioinformatical analysis, we identified two complementary DNA clones containing the coding sequence of histamine gated ion channels. The predicted proteins HisCl-α1 and α2 have all vital features shared by other ligand-gated ion channels. Application of histamine to *Xenopus laevis* oocytes injected with the cRNA of DM-HisCl-α1 or α2 activates a chloride current in a dose dependent manner that desensitizes upon prolonged application of agonist. The pharmacological properties of these homomultimeric channels are largely consistent with the properties of native histamine gated ion channels from invertebrates. In addition to homomultimeric channels, HisCl-α1 and -α2 form heteromultimeres that exhibit a high open-probability of about 50% in the absence of any agonist. Competitive antagonists of histamine like cimetidine act like a reverse agonist and close the population of open channels. Messenger RNA encoding the DM-HisCl-α1 receptor specifically localizes to the lamina region of the *Drosophila* eye, supporting the idea that DM-HisCl-α1 is a neurotransmitter receptor for histamine in the visual system. Together, the electrophysiological and pharmacological properties indicate that DM-HisCl-α subunits are the prototype of a new class of inhibitory ligand gated ion channels in invertebrates.

Antiobesity Evaluation of Histamine H3 Receptor (H3R) Antagonist Analogs of A-331440 with Improved Safety and Efficacy

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A-331440 {4'-[3-(3(R)-(dimethylamino)-pyrrolidin-1-yl)-propoxy]-biphenyl-4-carbo-nitrile} was previously shown (Faghih et al., 2004) to be a potent, selective H3R antagonist which lowered body weight in diet-induced obesity in mice. However, this compound yielded positive results in an *in vitro* micronucleus assay of genotoxicity. We synthesized and evaluated several modified biphenyl-nitrile analogs of A-331440 for H3R-related properties such as binding potency, selectivity and functional antagonist and/or inverse agonist efficacy. The best compounds were further evaluated for induction of genotoxicity in the micronucleus assay. Fluorinated analogs such as A-417022 (4'-{3-[(3*R*)-3-(dimethylamino)-1-pyrrolidinyl]propoxy}-3'-fluoro-1,1'-biphenyl-4-carbonitrile) and A-423579 (4'-{3-[(3*R*)-3-(dimethylamino)-1-pyrrolidinyl]-propoxy}-3',5'-difluoro-1,1'-biphenyl-4-carbonitrile) lacked genotoxicity in the micronucleus assay while maintaining equivalent or superior *in vitro* H3R properties compared to A-331440. A-417022 (30 mg/kg p.o., q.d., 28 days) reduced body weight in diet-induced obesity in C57/BL6 mice, comparably to A-331440 (30 mg/kg p.o., q.d.). A-423579 was also effective in obese mice (3 or 10 mg/kg p.o., b.i.d. for 14 days) causing dose-dependent reductions in body weight (5% and 10%) and in cumulative energy intake (16 and 28%) respectively. In diet-induced obese female Sprague-Dawley rats, A-423579 (10 mg/kg p.o., b.i.d. for 4 weeks) caused a 6.9% decrease in body weight while vehicle treated controls gained 3.7% over the same period. Significant decreases in cumulative energy intake, fat mass and plasma leptin were also observed. In free-fed rats habituated to spontaneously consume a high fat "snack" when presented, A-423579 (30 mg/kg p.o. single dose) caused a 30% reduction in 1 hr intake (6.7g compared to vehicle 9.6g) without affecting the normal behavioral satiety sequence. These data provide further support to the hypothesis (for review, see Hancock, 2003) that H3 antagonists may have therapeutic potential for the treatment of obesity and related disorders.

Faghih, R., Y.L. Bennani, M. Cowart, T.A. Esbenshade, G.B. Fox, K.M. Krueger, T.R. Miller, D.G. Witte, B.B. Yao and A. A. Hancock. Structure-activity relationships of A-331440: A new histamine-3 antagonists with anti-obesity properties, Inflammation Research (in press) 2004.
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Histamine mediated modulation of tumor progression marker expression in mice experimental dermatofibrosarcoma model

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The complex chemical and photocarcinogenesis is a classical experimental model for studying development of tumours. Histamine seems to be an immunmodulator and also could have an important role in angiogenesis regulation associated with promotion of tumour progression. In our study, we used transgenic mice genetically lacking histidine decarboxylase (HDC-/-), to evaluate the role of endogenous histamine synthesis on tumour growth and spreading. Invasion markers and in general the tumour development have been measured. To examine the alterations in expression of uPA, uPAR and VEGF their relative mRNA levels were measured from the tumour tissue by quantitative real time RT-PCR. Angiogenic markers as PAI-1/2, angiopoietin1/2, HGF, bFGF, and VEGFs were analysed by pathway specific gene expression profiling system (GEArray^{TM}Q Series Superarray KIT).

The chronic suberythemal UV-B radiation together with croton oil treatment accelerated CD34, CD44 positive dermatofibrosarcoma (DFS) genesis in this model. Tumour locations were similar in both wild type and transgenic animals, but the tumour incidence was significantly higher in HDC$^{+/+}$ mice. Controversially, the tumours appeared earlier in the HDC$^{-/-}$ mice and their survival were worse than the wild types.

The expression of uPA , uPAR and VEGF were significantly reduced in the HDC$^{-/-}$ mice, the H1R and H2R were unchanged and the array data showed elevated mRNA level of PAI-2.We have established in vitro culture from the tumours (DFS$^{HDC+/+}$ and DFS$^{HDC-/-}$) for further investigations.

In conclusion, we showed that DFS was induced with this carcinogenesis protocol on the histamine lacking, as well as, the wild type ice. Plasminogen activator system, and vascular endothelial growth factor could be involved in the tumour growth regulation by histamine mediated way.

Expression and cleavage of STAT1alpha is regulated via histamine (H4) receptor signalling

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The TH1/TH2 balance can be influenced by histamine, and allergic reactions are associated with excessive histamine production. The aim of this work is to study the intracellular downstream signalling of histamine receptor (H) modulated phosphoregulation in the so-called JAK-STAT pathway. PBMC from 6 non-atopics (20-28 years, 4 female, IgE < 100 IU) were stimulated with phytohemagglutinin (PHA) and incubated for 3 days. IL-4 and IFNgamma were measured by ELISA. Histamine, thioperamide and clobenpropit were added alone or in combination at 4 hours post-plating. The MTT-test was used to examine cell proliferation. Western blots were performed for determination of latent STAT1alpha and phosphorylated STAT1 (aSTAT1). In accordance with recent results, maximum IL-4 production was shown after one day cell culture and IFNgamma was increased after two days. The delayed IFNgamma production stimulated cleavage of the 119 kDa STAT1a (119-STAT1). A 28 kDa STAT1alpha (28-STAT1) fragment could be detected. In contrast to the latent STAT1a, the production of the phosphorylated 119-STAT1 was reduced while the levels of phosphorylated 28-STAT1 peptides increased over time of stimulation. In Western blot experiments it could be shown that histamine inhibited this degradation potency, and the H4 histamine agonist clobenpropit aggravated this process. Thioperamide did not show any pronounced effects. These data demonstrated that histamine has the potential to modulate the IFNg induced JAK/STAT pathway by cleavage of 119- STAT1 producing a 28-STAT1 peptide. Clobenpropit is considered to aggravate the responses of histamine via H4 transmembrane signalling.

Czeslaw Maslinski Symposium

Characterisation of native and recombinant histamine N-methyltransferase proteins

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Histamine N-methyltransferase (HNMT) catalyzes the transfer of a methyl group from S-adenosylmethionine (SAM) to the imidazole ring of histamine forming the biologically inactive compound N$^{\tau}$-methylhistamine. Although HNMT is expressed in most mammalian tissues, the enzyme is difficult to purify due to its low expression level and rapid inactivation. We developed a chromatographic purification scheme with affinity binding to immobilized histamine as the crucial step that facilitated the isolation of 20 µg homogenous HNMT from 20 g of porcine kidney cortex tissue with a 10% yield and a ca. 5000-fold enrichment. Porcine HNMT is a soluble monomeric protein of 33 kDa and shows K_m values for histamine and SAM of 9.8 µM and 8.3 µM, respectively. The enzyme is strongly inhibited by the reaction products N$^{\tau}$-methylhistamine and S-adenosylhomocysteine with K_i values of 4.1 µM and 14.6 µM, respectively. Among the strongest inhibitors of HNMT are the antimalarial drug amodiaquine (K_i = 40 nM), the SH-group reagents p-hydroxymercuribenzoate (K_i = 90 nM) and N-ethylmaleimide (K_i = 6.2 µM), and the histamine H_2 receptor antagonist dimaprit (K_i = 2 µM). Tryptamine (K_i = 2.6 µM) and serotonin (K_i = 30 µM) also efficiently compete with histamine methylation by HNMT. In order to obtain larger amounts of porcine and human HNMT, we used the cloned cDNAs to express these HNMT proteins as glutathione-S-transferase fusions in E. coli. The recombinant enzymes are fully active and have similar properties as their native counterparts. Homogenous recombinant human and porcine HNMT can be produced in mg-amounts for analytical and diagnostic applications.

Effects of nociceptin on neuronal and mast cell histamine in the brain.

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Nociceptin, an endogenous ligand for the opioid-like receptor (ORL_1 /NOP) from the brain, has been shown to reverse opioid analgesia in the brainstem and can produce anti-nociception or hyperalgesia at the level of the spinal cord in differing conditions [1]. Injections of histamine (HA) into the CNS produce powerful antinociception [2]; so HA has been suggested to be involved in the modulation of the nociceptin-induced pain transmission [3]. The question is, whether HA might participate in the nociceptin effect directly; or this opioid-like peptide might influence the release of HA (from histaminergic nerves or from mast cells in the brain).

Male Wistar rats (170-230g) were housed at room temperature under normal light cycle. Food was withdrawn 24 h prior to experiment. Nociceptin (Bachem) and the mast cell degranulating substance, 48/80 (Sigma) were given in a volume of 20 µl intracerebro-ventricularly (i.c.v.) at doses of 10µg/rat and 100µg/kg, respectively. Controls got only vehicle (saline). Under slight ether anaesthesia, blood (3ml) was gained through nasal canthus into K-EDTA containing vacutainers, and cerebrospinal fluid (120-150 µl) was drawn through the foramen occipitale magnum and collected into mini-sorb vials 1, 3, and 24 h after the treatment. Animals were sacrificed, the brain was removed, dissected and homogenised in phosphate buffer (pH 7.9). The plasma, the cerebrospinal fluid (CSF) and the supernatants of the brain homogenates were used for the enzymic radioactive measurements of HA. In the course of combined treatment nociceptin was given 1h before 48/80. Experimental procedures were done according to 86/509/EEC.

Nociceptin-treated rats showed significantly higher levels of HA, vs controls, in the CSF, the hypothalamus, the hippocampus and the cortex 3h after the treatment. 48/80-treated rats produced significantly higher levels of HA in the CSF and the hypothalamus, but not in the plasma, the hippocampus and the cortex 1h after the treatment. Combined treatment produced significantly higher HA levels in the CSF and the hypothalamus, than single treatment indicating additive effects of the compounds.

In summary, data showed that nociceptin increases HA concentrations in both the CSF and the brain parts tested (hypothalamus, hippocampus, cortex) probably by accelerating the release of histamine from histaminergic nerves and also from mast cells (located almost exclusively in the hypothalamus and the thalamus). This finding is in line with opioid µ-receptor agonist DAGO and morphine [4], but contradicts to that assumed for HA in nociceptin-induced pain transmission [3,5]. Thus, the present data may reveal a more complex modulatory role of the amine in nociceptin-induced pain-transmission.

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Evidence for histaminergic involvement in the action of modafinil

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Modafinil, (RS)-2-(Diphenylmethylsulfinyl) acetamide, is a novel wake-promoting drug used for the treatment of narcolepsy. However, the mode of action of modafinil is unclear. We previously reported that both peripheral (150mg/kg, i.p.) and central injection (1nmol, i.c.v.) of modafinil significantly increased hypothalamic histamine release in urethane-anesthetized rats, suggesting that modafinil activates the histaminergic system (1). However, since one hour was required to increase histamine release even by the central injection, the pharmacological effect of modafinil on the histaminergic system is probably delayed by anesthesia. Thus, in the present study, we investigated the effect of modafinil on the hypothalamic histamine release as well as locomotor activity in the freely-moving rats using in vivo microdialysis. Modafinil (150mg/kg, i.p.) significantly increases locomotor activity.This effect was observed immediately after the injection of drug and gradually reduced in 2 hours. Hypothalamic histamine release was simultaneously increased by drug administration, and the amount of histamine release correlated well with that of locomotor activity. Moreover, modafinil did not affect locomotor activity in the mice whose neuronal histamine was depleted by the injection of alpha-fluoromethylhistidine. These observations suggest that modafinil promotes waking via the activation of the histaminergic system, and that the histaminergic system plays a pivotal role in modafinils action.

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The role of peripherally acting histamine in metoprine-induced reversal of haemorrhagic shock in rats - skeletal muscle microcirculatory studies

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An increase in central histamine concentrations after loading with L-histidine [1] or inhibition of histamine N-methyltransferase (HNMT) activity [2] leads to the reversal of critical haemorrhagic hypotension in rats. The effect is due to a mobilisation of compensatory mechanisms, including an increase in the activity of the sympathetic nervous system and the renin-angiotensin system, and release of arginine vasopressin and proopiomelanocortin-derived peptides. The study was undertaken to examine the influence of peripherally acting endogenous histamine on the skeletal muscle microcirculation during the resuscitating effect of intraperitoneally administered HNMT inhibitor metoprine. Studies were carried out in male Wistar rats subjected to hypotension of 20-25 mmHg, which resulted in an increase in renal, hindquarters and mesenteric vascular resistance, decreases in regional blood flows, and the death of control animals within 30 min. HNMT inhibitor metoprine (15 mg/kg) administered at 5 min of critical hypotension produced increases in mean arterial pressure and heart rate, and a 100% survival at 2 h after treatment. The action was associated with a 3.4-fold increase in hindquarters blood flow (Transit Time Flowmeter Type 700; Hugo Sachs Elektronik, Germany) and 2.7-fold increase in skeletal muscle microcirculatory flow (Laser Flowmeter BRL-100; Bio Research Center Co, Ltd, USA), as measured 30 min after treatment ($P < 0.01$ vs. the control saline-treated group). Skeletal muscle concentration of histamine 20 min after metoprine treatment was 4.8-fold as high as in the control animals (3.01 ± 0.67 vs. 14.41 ± 2.99 μg/g of wet tissue; $P < 0.001$).

In conclusion, the results demonstrate that a decrease in skeletal muscle vascular resistance, with a rise in skeletal microcirculatory flow, during metoprine-induced resuscitating action may result not only from compensatory mechanisms-mediated mobilisation of blood from the reservoirs in the venous part of the circulatory system [3], but also from a direct action of endogenous peripheral histamine.

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Histamine levels in the rat hypothalamus under pathological conditions

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Brain histamine is localized in mast cells and neurons. We aimed at evaluating the relationship between rat hypothalamic histamine content and hyperthyroidism or experimental arthritis.

Male Wistar rats of 250-300g were divided into 5 groups, each subdivided into A and B. Groups 1A-2A and 3A were injected i.p. with 1mg/Kg of C48/80 once and qdx4d, respectively. Hyperthyroidism was induced in group 4A by sc injection of 0.25mg/Kg L-thyroxine qdx14d[1], while the arthritis group 5A received Freud's adjuvant id into the paw[2]. Groups 1B-5B served as controls, being treated with saline accordingly. Groups 1, 2, 3-4 and 5 were sacrificed 2h, 5d, 24h and 14d after the last dose. Following sacrifice and brain removal (license K/4130/02), the hypothalamic histamine was quantified fluorometrically[3] and expressed as mean±SEM% of the respective control. Statistical analyses were performed by ANOVA.

Histamine levels were reduced in groups 1A and 3A ($p<0.5$, n=3-6) compared to control, while no significant difference was detected between C48/80 treatments. Amine levels were significantly lower in hyperthyroid ($p<0.05$, n=3-9) or arthritis ($p<0.01$, n=10) rats, compared to control. No significant difference was located between control groups 1B-5B.

These data indicated that the hypothalamic histamine content might be affected by underlying disorders in a similar direction as C48/80. Whether this is attributed to a direct action on histaminergic neurons and/or brain mast cells remains elusive, although thyroid hormones have been reported to decrease histamine levels and mast cell number in the neonatal rat brain[4].

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The influence of histamine H3 receptor antagonists on IgE synthesis

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Previous investigations have been shown four pharmacologically distinct subtypes of receptors, i.e., the H_1R, H_2R, H_3R and H_4R receptors, which are all members of the G-protein coupled receptor (GPCR) family (Heron A., 2001, Nguyen T., 2001). The H_1R mediates most of the histamine effects in allergic reactions. Nevertheless other types of histamine receptors are involved in different pathological processes, including allergic reactions, different malignancies, brain activity e.c.. Previously it has been shown that histamine acting on H_1 and H_2 may be involved in the regulation of IgE synthesis in atopy and lymphoproliferative diseases (Khanferyan R., Riger N., 2000). The determination of H_3 and H_4 receptors on the membrane of mononuclear cells (Leurs R.et al, 1998, Oda T. et al, 2000) support the idea that these new types of histamine receptors are thought to be important for the regulation of IgE synthesis in allergic reactions. In this investigation the influence of two H_3-receptor antagonists, FUB 181 (rat: K_i = 13 nM) and Imoproxifan (rat: K_i = 0.26 nM) on IgE synthesis have been studied. Mononuclear cells (MNC) of healthy donors and allergic patients with ragweed sensitivity were cultivated in RPMI 1640 at 37 ^{0}C with and without FUB 181 and Imoproxifan in the range of concentrations from 10^{-5} to 10^{-8} M. The determination of the total IgE was done in the supernatants of 9-days culture of MNC by CAP FEIA method (Pharmacia). The level of the key IgE regulatory cytokine IL4 was measured in the supernatants of 3-days culture of MNC by ELISA using IL4 immunoenzyme kit (Diaclon). It has been shown that both antagonists have similar effects on IgE synthesis in MNC of healthy donors as well as in allergic patients but IgE synthesis increased more in the last group ($p<0.001$). It has been shown that Imoproxifan stimulates IgE synthesis in all studied concentrations in MNC culture of healthy donors as well as in allergic patients (from 1.5 to 1.7 fold). At the same test system only high concentrations of FUB 181 (10^{-5} and 10^{-6}M) showed weak increasing effects on IgE-response in MNC of healthy donors and well-pronounced effects in atopic patients. The increased synthesis of IgE after the H_3receptor blockade was IL4-dependent. As has been shown that high concentrations (10^{-5} M) of Imoproxifan produced an increase in the IL4 levels from stimulated MNC supernatants. This effect was more pronounced in allergic patients. The production of IL4 in this group was more than 3 fold (from 3.62 to 10.32 pg/ml) higher. The lower enhance in IL4 production ($p<0.05$) was in healthy donors. Thus, H_3 receptors may be involved in the regulation of IgE synthesis both in healthy donors and allergic patients and this effect is IL4-dependent. The level of IgE stimulation induced by H_3-receptor antagonists may be due to differences in the potency of H_3-receptor antagonists.

LITERATURE

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Identification of 4-(1*H*-Imidazol-4-yl-methyl)pyridine (Immethridine) as a novel potent and highly selective histamine H_3 receptor agonist

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In the past, many histamine analogs have been developed as selective H_3 receptor agonists. For ligands with a basic nitrogen in the side chain, an increased rigidity often results in an increase in binding affinity and agonistic potency at the human H_3 receptor. The piperidine side chain analog, immepip, is a typical example of such a ligand which exhibits a 10-fold higher affinity at the human H_3 receptor than its flexible congener imbutamine. Immepip is currently one of the most potent H_3 agonist known (pK_i = 9.32 and pEC_{50} = 9.88 at the human H_3 receptor), but also shows reasonable potency for the recently discovered histamine H_4 receptor (pK_i = 7.66 and pEC_{50} = 7.25).

In our search for potent and selective histamine H_3 and H_4 ligands, a further conformational constraint in the side chain was achieved by a replacement of the piperidine ring with pyridine rings. None of compounds in the new series exhibits an increased affinity and functional activity at both the human H_3 and H_4 receptors. At the human H_3 receptor, the 4-pyridinyl analog of immepip, immethridine, acts as a full agonist with high affinity and potency (pK_i = 9.07 and pEC_{50} = 9.74). Although the affinity of immethridine at the human histamine H_3 receptor is equal to the affinity of immepip, it displays a 10-fold lower binding affinity at the human H_4 receptor (pK_i = 6.61 and pEC_{50} = 6.04). This decreased binding affinity of immethridine at the human H_4 receptor leads to a 300-fold selectivity at the human H_3 receptor. Immethridine is therefore identified as one of the most potent and selective agonists for the human H_3 receptor.

Plasma diamine oxidase during continuous administration of heparin in thrombosis patients

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Besides its anticoagulant activity, the sulfated polysaccharide heparin has numerous other biological effects. Especially the anti-inflammatory and immunoregulatory properties of heparin may be associated with its ability to release the histamine degrading enzyme diamine oxidase (DAO) from tissue-bound sites into the circulation. Whereas DAO activity is at the limits of detection in normal human plasma, application of heparin leads to a significant increase of plasma DAO activity, but previously only the effect of bolus injection of unfractionated heparin had been studied. To investigate DAO release during continuous heparin infusion, 28 patients with deep vein thrombosis (DVT) undergoing heparin therapy were analysed. Whereas continuous heparin infusion did not lead to any increase of plasma DAO activity in 12 patients (43%), 6 patients (21%) showed a single elevated and 10 patients (36%) permanently elevated plasma DAO activity. The groups of patients exhibiting different DAO release responses did not differ in age, sex, body weight, concomitant diseases, heparin infusion rates, coagulation indices, location and extension of thrombosis, or clinical outcome. However, the rate of idiopathic DVT was significantly higher in the group of patients releasing DAO. This study shows for the first time that continuous heparin infusion can lead to DAO release and that individuals exhibit considerable differences in their release response. Although the significance of heparin-induced DAO release needs further clarification, our results indicate that postheparin plasma DAO activity could be an interesting parameter correlated with idiopathic DVT.

Expression of histamine degrading enzymes in porcine tissues

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Histamine can be inactivated either by diamine oxidase (DAO) catalyzed oxidative deamination or by histamine N-methyltransferase (HNMT) catalyzed ring methylation. Although these enzymes and their properties have been known for quite some time, comprehensive data on the tisssue- and cell-specific expression of DAO and HNMT were not available. Therefore, we collected samples from most tissues of the pig to evaluate the expression of the histamine degrading enzymes by both activity measurements of cell homogenates and reverse transcription-PCR of total cellular RNA. In accordance with previous results, we found DAO to be expressed predominantly in the intestine, the kidney, and the spleen. Besides, DAO activity or mRNA was detected in only a few other tissues such as lymph node and eye. In contrast, HNMT was found to be expressed in all tissues analyzed and comparable HNMT activity was measured in most tissue homogenates. Whereas DAO as a secreted enzyme might be important for the inactivation and scavenging of extracellular histamine at only a few sites, HNMT as a cytosolic enzyme might have to deal with intracellular histamine almost everywhere. The presence of a constitutive intracellular histamine inactivation capacity implies that histamine is an important signalling molecule in most tissues.

Effects of moderate stress on plasma histamine levels in laboratory dogs

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Plasma histamine is known to be increased in rats after stressful manipulations. The aim of this study was to determine whether plasma histamine levels in dogs would change in a similar way, and if histamine could be used as an indicator of stress response in laboratory dogs. As an exact parameter referring to stress plasma cortisol levels were measured. (I) Blood was sampled from laboratory dogs (Foxhound-Boxer-Labrador Retriever-Mix; n=6) at seven times over 24 hours. (II) These dogs were exposed to an unfamiliar environment. Blood samples were taken before and after the impact of the presumed stress. (III) The dogs were not fed for two days whilst they could see food preparation and feeding of kennel neighbours. Blood was taken during expectation of food and 30 minutes later. Analytical procedures: After ion-pair extraction with bis(2-ethyl-hexyl)phosphoric acid (B2EHPA) plasma histamine was determined by HPLC using a precolumn derivatisation with o-phtaldialdehyde as the fluorescent agent. Plasma cortisol was measured by a luminescence immunoassay (IBL, Hamburg).

There was no significant variability over 24 hours visible neither in histamine nor in cortisol levels. Expectation of food and hunger caused no changes in cortisol and histamine levels. Plasma cortisol levels increased significantly ($P<0.05$) after exposure to a foreign environment (mean±SEM: 37.3±5.7 vs. 107.2±18.1 nmol/l plasma), but histamine levels showed no significant alteration (mean±SEM: 9.0±1.3 vs. 8.5±1.2 nmol/l plasma).

In our investigations histamine as well as cortisol levels showed no circadian rhythm over 24 hours. Moderate stress as applied in this study seems to have no effect on histamine levels in laboratory dogs. Even though a significant increase in cortisol after confrontation with a new environment suggests that a stress response has been taking place, histamine levels still stayed unaltered.

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Dual effects of pyrilamine on the absence seizures in the WAG/Rij rats

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The rats of the WAG/Rij strain are widely used as a model of human absence epilepsy [1]. Recently, we reported changes in the brain histaminergic system of the WAG/Rij rats, on the level of tissue concentrations of histamine and expression of H1 receptors [2,3]. In the present study, it was checked whether systemic administration of an H1 receptor antagonist had effect on absence seizures in WAG/Rij rats. For this purpose, 10 male WAG/Rij rats (weight 220-350g) were chronically implanted with epidural cortical electrodes, and injected with pyrilamine (20 mg/kg i.p). Absence seizures were scored from EEGs of freely moving animals, under the baseline conditions (1h) and after the treatment (1.5 h.). Statistical analysis (ANOVA) revealed, that in the rats with low basal level of absence seizures (1.3±0.8% of recording time) pyrilamine facilitated the seizures (treatment effect: Mann-Witney test, N=5; $p<0.011$); whereas in the rats with high incidence of absence attacks in the background EEG (8.0±2.6%) pyrilamine decrease the seizures (treatment effect : Mann-Witney test, N=5, $p<0.009$) [difference in the basal seizure level between the rat groups: $F(1,8)=30.96$, $p<0.0005$]. In 7 out of 10 rats, the pyrilamine injection was repeated after 4-6 days, and the effects were along the same lines.

The results obtained in the present study, might indicate that the rats with high and low severity of absence epilepsy differ in respect to the brain histaminergic functions. However, the possibility of different sensitivity of rats of these 2 groups to non-specific effect of pyrilamine can not be excluded. Further studies are needed to clarify the question.

Histamine excites noradrenergic neurons in locus coeruleus in rats.

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Histamine is implicated in the control of many brain functions including arousal. Histaminergic neurons send dense projections through the entire CNS, including the locus coeruleus (LC) - the main noradrenergic nucleus in the brain that also participates in the control of arousal. In this study we have examined the effect of bath-applied histamine on cells in the LC and the expression of histamine receptors in this area. Coronal slices were prepared according to standard techniques. Single-unit extracellular recordings were performed using glass pipettes filled with 2M NaCl (3-10 MΩ). Total cellular mRNA was isolated from LC slices and the expression of histamine receptors was investigated by RT-PCR.

Noradrenergic neurons showed slow (1.5 – 2.5 Hz) regular firing. Bath application of histamine (10 μM) increased the firing of NA cells to 126,7 ± 10,2% of control. This effect was dose-dependent – the application of 100μM histamine led to larger increase of firing frequency – up to 424,3 ± 79,5% of control. An RT-PCR study revealed the presence of H1, H2 and H3 receptors in LC. All 3 receptors were expressed in two other aminergic nuclei – substantia nigra and the ventral tegmental area as well.

These findings suggest that the histamine-induced arousal could be partly mediated via the excitation of noradrenergic neurons in locus coeruleus.

The neuroprotective effect of the central histaminergic neuron system on kainic acid-induced neuronal death in the developing hippocampus in vitro

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Epilepsy causes neuronal damage in particular in the hippocampal area of the brain. Histamine regulates seizure threshold through H1 and H3 receptors. The H3 receptor activation inhibits glutamate release by inhibiting presynaptic calcium entry (Brown et al.1999), and the H1 receptor affects e.g. potassium efflux. Kainic acid (KA)-induced seizures are one of the most commonly used epileptic models both in vivo and in vitro. In this study, we used a co-culture system consisting of organotypic slices of the hippocampus and the posterior hypothalamus, which contains histaminergic neurons, to clarify the role of the central histaminergic system in KA-induced neuronal death in the developing hippocampus. Histaminergic fibres were visualised immunocytochemically, and Fluoro-Jade staining was applied to detect degenerating neurons. The results showed that histaminergic neurites grow into the hippocampus in vitro. After KA treatment, no significant changes were observed in histaminergic innervation within the hippocampus. Moreover, neuronal death was significantly decreased in the hippocampal subareas CA3a/b and dentage gyrus after KA treatment for 12 h when cultured together with the posterior hypothalamus. No decrease in hippocampal neuronal death was observed when hippocampus was cultured together with slices of the anterior hypothalamus, which did not contain histaminergic neurons. Finally, KA-induced neuronal death significantly and dose-dependently increased in the presence of H1 receptor antagonist triprolidine in hippocampal slices cultured together with the posterior hypothalamus.

Our conclusion is that the central histaminergic neuron system could protect the developing hippocampus from KA-induced neuronal death in this co-culture system, which was used as an in vitro epileptic model. Furthermore, our results indicate that the neuroprotective effect of histamine may at least partly be mediated through H1 receptors.

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Inhibition of Mast Cell Histamine Release by Specific Phosphodiesterase Inhibitors

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Mast cells play a crucial role in allergic diseases through the release of inflammatory mediators such as histamine. Non-selective phosphodiesterase (PDE) inhibitors are long known to inhibit mediator release from both rat and human mast cells. However, despite a growing number of PDE isozymes currently identified, the exact nature of the PDE isozymes regulating the activities in mast cells remains uncertain.

In the current investigation, we first characterized the PDE isozymes involved in regulating mediator release in mast cells by studying the effects of a range of isozyme-selective PDE inhibitors (for PDE-1 to PDE-5) on the IgE-mediated histamine release from rat peritoneal mast cells (RPMCs). In general, selective inhibitors of the PDE-1, -2 and -5 isozymes had no significant effect while selective inhibitors of the PDE-3 and -4 isozymes demonstrated concentration dependent inhibition of histamine release. PDE-4 inhibitors were more potent: for instance, the PDE-4 selective inhibitor rolipram reduced the anti-IgE-induced histamine release by about 30% at 10 □M whereas only around 20% inhibition was observed with most of the PDE-3 selective inhibitors at this concentration. The degree of inhibition achieved by these selective PDE inhibitors was, however, mild when compared with the non-selective PDE inhibitors tested, such as theophylline, which inhibited histamine release by a maximum of 98%. Hence, we next investigated if combination of PDE-3 and -4 inhibitors would produce a more complete inhibition of histamine release. Consequently, it was observed that a combination of rolipram (10 □M) and the PDE-3 inhibitor siguazodan (1 □M) produced a significantly higher level of histamine release inhibition (50.0% $\pm$ 7.1%) than the expected inhibition (33.1% $\pm$ 5.0%).obtained by adding the levels of inhibition observed when mast cells were incubated with individual inhibitor separately.

Our results suggest that PDE-3 and -4 are the major PDE isozymes involved in the regulation of mediator release in mast cells and a synergistic inhibitory effect can be obtained when the activities of these two isozymes were suppressed by a combination of specific PDE-3 and PDE-4 inhibitors. However, the enhanced level of inhibition is still not as high as that obtained with theophylline.

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Notes

How to bind and activate histamine H_1 receptors?

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With the availability of the amino acid sequence of drug targets of interest medicinal chemists are trying to rationalize drug-protein interaction in order to come to a structure-based drug design approach. For the super-family of G protein coupled receptors (GPCRs), which is responsible for the cellular communication by many signaling molecules including histamine, this approach has so far proven to be quite difficult. One of the underlying difficulties has for many years been the lack of suitable structural data in order to derive high quality models for receptor-drug interaction.

The elucidation of a X-ray structure of the light sensitive GPCR rhodopsin in 2000 is a milestone in the area of GPCR research and has led to the generation of new models for GPCR proteins. In this presentation I will highlight the use of this information and the application of site-directed mutagenesis in order to obtain ideas on the interaction of agonists and antagonists with the histamine H_1 receptor. Moreover, we have recently obtained detailed information on a potential mechanism of activation of the histamine H_1 receptor.

Hiroshi Wada Memorial symposium

From rat liver [^{3}H]mepyramine binding to solid state NMR measurements of the histamine H_1 receptor

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For medicinal chemists rationalization of drug-protein interaction is essential for a rational design of new, biologically active ligands. For many years our laboratory has had an interest to develop models for the ligand receptor interaction at the histamine H_1 receptor. In the mid eighties, we got interested in the potential use of rat hepatocytes as cellular model for the H_1 receptor. This interest was stimulated by a reported huge amount of [^{3}H]mepyramine binding sites in rat liver membranes. In a detailed study with a variety of stereoselective H_1 antagonist we showed that the [^{3}H]mepyramine binding site did not fit the known H_1 receptor pharmacology. Moreover, in collaboration with profs Wada and Fukui, who were investigating the use of purified [^{3}H]mepyramine binding protein from rat liver as a means to clone the H_1 receptor gene, we could show that the [^{3}H]mepyramine binding protein represented a cytochrome P450 isoform. Years later investigations on [^{3}H]thioperamide binding to rat brain membranes led to a similar conclusion, highlighting that caution should be taken when performing radioligand binding in tissue preparations.

Using bovine adrenal gland, instead of rat liver, profs Wada and Fukui succeeded eventually in cloning the bovine H_1 receptor, allowing the histamine research community to study the molecular aspects of H_1 receptor action. In a collaborative effort with profs De Grip and de Groot (Leiden) recently succeeded in the purification of the human H_1 receptor and we obtained the first structural data on the ligand-receptor interaction by solid state NMR measurements.

Activity modulation of the human histamine H_4 receptor by hydrophobic group-containing ligands

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Several research groups have recently reported the potential of the histamine H_4 receptor (H_4R) as a drug target for immune system-related disease (Buckland *et al.,* 2003 and references therein). Potent and selective H4 receptor ligands are needed to further characterize the receptor. Our study is aimed to elucidate structural requirements for H_4 receptor ligands in order to facilitate rational drug design. We have evaluated various classes of compounds that bear different hydrophobic groups for their affinities and functionalities for the human histamine H_4 receptor (hH_4R) stably expressed in SK-N-MC cells. The results reveal interesting structure-activity relationships, and new lead compounds for obtaining potent and potential selective H_4R ligands have been identified.

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Histamine and orexin neurons: synergistic and complementary regulation of the sleep wake cycle?

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The posterior hypothalamus, classically recognized as an important structure for waking, contains several neuronal populations. Our studies in cats support the hypothesis that this role is mediated, in part, by the widespread projecting histamine(HA)neurons. The recent identification of orexin cells adjacent to HA neurons strengthens the idea that multiple neuronal populations are involved in the hypothalamic mechanisms with regards to sleep-wake control. Indeed, orexin deficiency seems to be responsible for the pathogenesis of narcolepsy and like HA neurons, orexins containing cells may be involved in arousal by their widespread projections. Recently, histidine-decarboxylase (HDC, HA-synthesizing enzyme) and orexin knockout(KO) mice have been developed, allowing the respective role of each of these systems to be investigated. Thus, HDC-/-mice exhibited permanent change in 1) cortical-EEG: decrease in theta rhythm during waking and in the slow wave sleep/waking power ratio 2) sleep-wake cycle: increase in paradoxical sleep (PS) and deficit of waking around lights-off, and 3) behavior: signs of sedation when faced with various stimuli, e.g., environmental change. These effects are likely to be due to the lack of HA, as the KO mice showed no HA-immunoreactive neurons and no response to HA related agents. These results demonstrate the importance of HA neurons in the qualitative aspects of waking and in maintaining the brain awake faced with behavioral challenges. (Parmentier-et-al.,2002, J.Neurosci.). Orexin-KO mice share some phenotypes of HDC-/-mice, e.g., obesity and increase in PS. However, the PS increase in HDC-/-mice was seen during the light-period, whereas that in orexin KO mice occurred during darkness and is accompanied by narcolepsy (Chemelli-et-al.,1999, Cell). The latter phenomenon was not seen either in HDC-/-mice or in normal animals treated with anti-HAergic agents. Whereas the correlation between the obesity and PS increase in each genotype of KO mice remains to be determined, these data suggest that the hypothalamic mechanisms controlling body weight and PS are multiple and include both HA and orexin neurons, which might exert a complementary/permissive control over PS.

Histamine, a bad boy or a good girl in kainic acid-induced epilepsy?

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In this study we used systemic kainic acid (KA) administration to produce status epilepticus (SE)-induced brain damage in rats, to investigate if histamine contributes to seizures and neuronal damage or protects neurons in this animal model.

Decreased brain histamine content was produced by systemic α-FMH 6 h before KA injection. 25 rats were followed by EEG recording after implantation of 2 cortical electrodes and by video monitoring before collecting the brains 16 h after KA injection for morphological analysis. Rats treated with α-FMH+KA had increased seizure susceptibility when compared to those treated with saline+KA, as shown by the higher number of animals that reached SE state, confirming the previously published data from electrically or chemically induced seizures. However, there were no differences in EEG (time to the appearance of the first spike, total number of spikes or time to the end of continuous spiking activity) between α-FMH+KA- and saline+KA-treated rats. Also, the time to the occurrence of first generalized seizure or to the onset of continuous behavioural seizure activity did not differ between the groups. How the decreased brain histamine content affects neuronal damage caused by KA was analyzed with immunohistochemistry of a dendritic microtubule-associated protein-2 (MAP-2) and with *in situ* hybridization of a cytoplasmic antioxidant enzyme Cu/Zn-superoxide dismutase (Cu/Zn-SOD). Many α-FMH+KA-treated rats had less damage than saline+KA-treated rats in the piriform cortex and 2 amygdaloid areas when MAP-2 was used as a damage marker. α-FMH-pretreatment did not have any effect on KA-induced damage when Cu/Zn-SOD was used as a damage marker.

These results suggest that brain histamine does not protect against neuronal brain damage in this animal model, although it protects against the convulsion by affecting the seizure threshold level.

Histamine and IL-6 interactions in the stimulation of nerve growth factor secretion from cultured astrocytes

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Histamine plays a key role in the modulation of inflammatory and immune responses. In this processes it interacts with numerous cytokines and neurotrophins, like IL-1, IL-2, IL-4, IL-6, etc. We have shown in our previous studies that histamine and IL-6 are both able to stimulate neurotrophic factor (NGF) secretion from cultured astrocytes [1, 2]. In the present work we were further investigated the possible interactions between histamine and IL-6 in this process. As experimental model, we used primary cultures of rat neonatal cortical astrocytes, prepared from the brain of Wistar rats [3]. The cells were treated with different concentrations of histamine, IL-6 or both, for 24 hours. In the further experiments, we pre-treated the cells with different concentrations of histamine for 24 hours, and than incubated them in the presence of IL-6 for the next 24 hours. Released NGF was determined in the culture medium by NGF-ELISA.

The results showed that addition of IL-6 (10 ng/ml) significantly increases histamine stimulated NGF secretion from cultured astrocytes. The enhancement of NGF secretion is particularly evident from the cells, which were pre-treated by 100 nM of histamine for 24 hours, in comparison to the NGF secretion, evoked by either histamine or IL-6 alone. Our data suggest that the influence of histamine on the NGF secretion from astrocytes is complex process, which, in a view of the long-lasting effects, involves also the interactions between histamine and IL-6.

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Expression of the histamine H4 receptor during rat development

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Expression of the H4 receptor has recently been revealed in peripheral tissues (e.g., immunocompetent cells). Here, we present evidence suggesting that the histamine H4 receptor is expressed in various embryonic tissues and the adult brain. Using a radiolabelled oligonucleotide probe designed to specifically recognize mRNA corresponding to a portion of the third intracellular loop of the H4 receptor, we detected expression of the H4 receptor in various rat tissues of embryonic stages E14, E16, and E19. We verified our *in situ* hybridization data with RT-PCR and by subsequently cloning the third intracellular loop of the H4 receptor from E18 olfactory epithelium, E18 brain, adult spleen, adult hippocampus, adult piriform cortex, and adult frontal cortex. From *in situ* hybridization data we ascertained that noteworthy embryonic expression is detectable in thymus, brain (cortex, caudate-putamen, and cerebellum), spinal cord, and olfactory epithelium. Regions in the rat adult brain with clearly detectable expression levels were the hippocampus and the piriform cortex. The cerebral cortex, thalamus, and caudate-putamen had low expression levels. We believe that a majority of the signal emanating from the CNS is neuronal; the signal emanating from peripheral tissues could be from migrating, developing hematopoietic cells. Previous failed attempts to detect H4 receptor expression in the adult brain may be due to the fact that expression in adult brain is much lower than during development. Given that the H4 receptor has been suggested to contribute to mast cell migration, the H4 receptor may play a similar role in developing neurons.

Czeslaw Maslinski Symposium

Histidine decarboxylase activity and histamine content correlate with prostaglandin E_2 production and tumor stage in human colorectal cancer

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Both histidine decarboxylase (HDC) activity and histamine content are increased in several types of tumors. The aims of this study was to evaluate HDC activity and histamine content in human colorectal specimens and determine whether these parameters correlate with tumor stage features, intratumor microvessel density (MVD) assessed as CD31 immunostaining, vascular endothelial growth factor (VEGF) expression and prostaglandin E_2 (PGE_2) production.

Tissue samples were obtained from 33 consecutive patients who had undergone surgical resections for primary sporadic colorectal tumors at the Department of General Surgery, University of Florence, Italy. Cancer tissue and adjacent normal mucosa were excised from each surgical specimen. The extent of VEGF immunostaining was recorded semi-quantitatively using a 3 grade system. HDC activity was assessed by measuring [^{14}C] CO_2 evolved from L-[carboxyl ^{14}C]-histidine (46 mCi $mmol^{-1}$). Histamine content was assessed fluorimetrically. PGE_2 production was assessed using a specific radioimmunoassay. Twenty-eight tumors were classified as adenocarcinomas and 5 tumors were classified as mucinous carcinomas . Tumors were classified into four stages according to the AJCC cancer staging system. When all the cases were considered, the mean value of MVD was 26.2 (± 2.3). Most of the tumors showed extensive staining for VEGF: 7 tumors (21.2%) were grade 0, 12 (36.4 %) were grade 1 and 14 (42.4 %) were grade 2. HDC activity, histamine content and PGE_2 production were significantly higher in the tumor specimens than in the corresponding normal mucosa (42.7±2.4 vs 15.8±1.0, p=0.003; 28.1±2.4 vs 0.7±0.3, $p<0.0001$ and 10.5±0.9 vs 3.1±0.2, $p<0.0001$, respectively). HDC activity and histamine content were significantly higher in tumors with lymph node and/or distant metastases (stage III-IV) than in those without any metastases (stage I-II) (51.51±3.1 vs 35.1±2.7, $p<0.001$ and 35.0±3.1 vs 22.4±2.0, p=0.03). Moreover, these parameters significantly correlated with PGE_2 production ($r_s=0.42$, p=0.01 and $r_s=0.30$, p=0.04). Neither HDC activity nor histamine content correlated with MVD or VEGF immunostaining.

Our data showed an increase in HDC activity and histamine content in colorectal cancer when compared with normal colonic epithelium. These findings suggest that histamine may play a relevant role in determining colorectal cancer growth and aggressiveness with a possible modulation of COX2 activity by histamine.

Post-infectional distribution and phenotype of mast cells penetrating human brains.

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Variation in proteinase content is a major feature of mast cells (MC) heterogeneity. Recently published data suggest, that two major subsets of mast cells: MC_T (tryptase phenotype) and MC_{TC} (tryptase/chymase phenotype) are selectively expanded in inflamed tissues depending on the acute or chronic inflammatory stage of the disease.

In the human central nervous system (CNS) the resident MC are the MC_{TC} phenotype, but phenotypes of MC infiltrating inflamed areas of CNS following different infections are not known. This study is the first report of MC distribution, activation, and phenotype observed in autopsy brains of individuals infected with bacteria, viruses or parasites.
In tissue sections MC were detected using monoclonal tryptase and chymase antibodies. All MC contained immunoreactive tryptase. Dual immunolocalization studies were used to detect MC tryptase and chymase in the same tissue sections. Examination showed that the MC_T phenotype predominated over the MC_{TC}, and the former represented approximately 95 % of the total MC in the inflamed brain sections. In early stage of inflammation diffuse rather than granular immunoreaction of tryptase suggests that MC activation/degranulation was not uncommon, especially when such findings corresponded to clinical observations. In contrast to the inconsiderable increase number of MC recruited to the brain following virus or bacterial infections, all brains infested by cysticerci or *Toxoplasma gondi* showed accumulation of numerous mast cells. A striking feature of our observations of MC distribution in neurocysticercosis was the occurrence of variable focal accumulations of these cells in the vicinity of cysticerci. In the congenital toxoplasmosis MC were scattered throughout the brain and meninges.

Our observations lead to the conclusion, that the MC_T phenotype is the main subset of mast cells participating in the brain inflammatory responses and that in brains infected by parasites numerous MC participate in host-defense reaction.

Czeslaw Maslinski - a founder member of the Histamine Club - EHRS

Czeslaw Maslinski, Professor of Pathophysiology, an honorary member of the EHRS since 1998, died on 11th July 2002 at the age of 81 years. He was one of the founder members of the EHRS and organized the first informal meeting of histaminologists in Lodz in 1971, which was the inspiration for the creation of the EHRS. The first formal meeting of the then "Histamine Club" was in Paris in 1972. He organized the 1978 meeting of the EHRS in Lodz and was a central figure in the 1998 Lodz meeting. He was responsible for promoting histamine research in Poland and was the Founder, President and then later Honorary Member and Honorary President of the Polish Histamine Research Society.

He graduated from the University School of Medicine Lodz in 1950 and obtained his MD in 1951 and his PhD in 1956. After a series of appointments in Warsaw, culminating as Professor of Pathophysiology and Head of Section of Experimental Pathophysiology at the Centre of Experimental Medicine, Polish Academy of Sciences, Warsaw; he returned to Lodz. Initially he was Head of the Department of General Pathophysiology Lodz University School of Medicine and then Head of the Department of Physiology, Polish Academy of Sciences Lodz. In 1970, he became Professor in the Institute of Biogenic Amines, Polish Academy of Sciences Lodz, where he stayed until 2000, between 1971 and 1991 he was Chairman of the Institute.

His research concentrated on two main areas. He was interested in phenomena connected with non-specific resistance to tuberculosis, e.g studies on the influence of thyroid function on the development of tuberculosis and the effect of tuberculosis on thyroid function and secondly in the metabolism and physiological functions of histamine and the pathogenesis and mechanisms of anaphylactic and allergic reactions. Within the second area, he examined topics such as general adaptation process in models of adaptation to histamine; the effect of histamine on collagen formation and polymerization in wound healing; the role of histamine in fast developing tissues normal and neoplastic; the role of histamine in pathogenesis of some metabolic diseases, hepatic encephalopathy. His latest studies, carried out at a time when most would expect to be retired, focused on the possible roles of histamine in physiology of the mammary gland.

He published over 200 papers in international journals. He obtained fellowships twice from the CRNS (France) and once from the WHO. He had numerous awards e.g. from the Ministry of Health (1970); 3 prizes from the Scientific Secretary of the Polish Academy of Science (1972; 1973; 1992); the Medal XXV Years of the Polish Academy of Sciences (1984), for his significant contribution to the development of Polish science; the Nicolaus Copernicus Medal, which is the highest award of the Polish Academy of Science (1995) for his scientific achievements. He was a visiting professor at many medical research institutes and laboratories in Europe, USA and Canada. He had 34 doctoral students and 7 candidates for habilitation.

Despite his failing eyesight, he continued to make contributions to scientific discovery and scientific meetings. He was passionate about his research and also about encouraging younger scientists to develop to their full potential. He fathered 2 sons and has left 3 grandchildren.

Expression of copper amine oxidases in porcine tissues

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Recently we characterized four porcine genes encoding members of the copper amine oxidase family. These include AOC1, an ortholog of the histamine inactivating enzyme diamine oxidase (DAO), AOC2, an ortholog of retina amine oxidase (RAO), AOC3, an ortholog of vascular adhesion protein-1 (VAP-1) implicated in lymphocyte adhesion, and AOC4, a soluble ortholog of VAP-1 (sVAP-1). In order to learn more about the localization and possible function of these enzymes we collected samples from most tissues of the pig and employed enzymatic activity measurements and reverse transcription-PCR with gene specific primers to analyze the tissue- and cell-specific expression of the four AOC proteins. AOC1/DAO was found to be expressed in gut, kidney, spleen, lymph nodes, and in the eye. AOC2/RAO expression was not confined to the retina but was also detected in the liver and a few other tissues. AOC3/VAP-1 was found to be expressed at low levels in many tissues, which might be explained by its presence in ubiquitous vascular endothelial cells. In contrast, expression of the soluble form AOC4/sVAP-1 was restricted almost exclusively to the liver where it might be secreted into the bloodstream to constitute soluble plasma amine oxidase. Detailed knowledge of the tissue and cellular distribution is a prerequisite to identify possible substrates and to deduce likely functions especially of the newly characterized AOC proteins. Apart from AOC1/DAO that has been shown to convert histamine and other diamines, it is presently not known if one of the other AOC homologs is involved in oxidative histamine metabolism.

Pre-clinical evaluation of novel H3 receptor antagonists

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Histamine H3 receptor antagonists have been shown to increase the release of neurotransmitters such as acetylcholine and histamine in the brain, and to enhance cognition in a number of rodent models. Several H3 antagonists are currently being developed as symptomatic agents for the treatment of cognitive deficits in conditions such as Alzheimer's Disease and Schizophrenia. To identify novel H3 antagonists, the following approaches were used. Compounds exhibiting high functional potency and selectivity for the human recombinant H3 receptor, and high binding affinity for the rat cerebral cortex H3 receptor, were rapidly progressed to in vivo studies to gain an early indication of oral activity and brain penetrance. Rats were dosed orally by gavage and brains were removed 3 hours later. Antagonists inhibiting ex vivo cortical [3H]-alpha-methyl histamine binding by >75% were further investigated in dose response studies. Potent antagonists showing ex vivo binding ED50 < 0.5mg/kg (at 3 hours) and appropriate brain and blood concentrations, were then tested for their ability to reverse R-alpha-methyl histamine-induced drinking in rats. Functionally potent compounds with appropriate pharmacokinetic profiles were then tested in the rat novel object recognition model of recognition memory, where they were assessed for their ability to reverse cognitive deficit in a 48h delay paradigm. Our experiences with these approaches to characterise novel H3 receptor antagonists will be discussed.

Histamine is a selective protector against cellular damaged produced by ionizing radiation

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The present work investigated the capacity of HA to modulate the oxidative damaged produced by ionizing radiation. In order to study the effect of histamine (HA) on radiosensitivity of transformed cells, MDA-231 (human breast carcinoma cells) were irradiated in vitro with doses ranging from 0 to10 Gy employing a 137Cs source of 189 TBq (Dose rate: 7.7 Gy/min). HA from 0.1 to 10 mM was added to cultures 20 hs before irradiation. The survival curves were adjusted using the Lineal-Quadratic model. The levels of the free radicals superoxide (O2·-) and hydrogen peroxide (H2O2) were determined by flow citometry employing specific fluorescence staining. The activity of the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) was also determined in MDA cells treated with HA 10 mM and/or irradiated with 2Gy. HA treatment produced a significant dose dependent decrease in survival of irradiated cells ($p<0.001$) clearly showing that it enhances radiosensitivity. The survival fraction at 2Gy was 0.24±0.03 for control and 0.11±0.02 for HA-treated cells. This effect of HA was correlated with a significant increase in H2O2 and in SOD activity, while O2·- levels and GSH-Px showed minor changes. To study a possible protective effect of HA on small-intestine and bone marrow, two groups of 10 mice were irradiated with a single dose of 10 Gy on whole-body. HA-group received a daily sc injection (0.1 mg/kg) starting 20 hs before irradiation. All animals were sacrificed 5 days after irradiation. Intestinal mucosae of 10 Gy irradiated HA-treated mice showed mild mucosal atrophy with conservation of villous projections and absence of edema, cellular preservation of nuclear and cytoplasmic characters. The number of crypts was 35 % higher than irradiated control animals. Similarly, bone marrow of HA group showed mild hipocellularity. The present data indicate that histamine can enhance radiosentivity of malignant cells and in addition protects normal tissues from high doses of ionizing radiation. This selective effect is exerted in part by the modulation of antioxidant enzymes.

Distribution of the H1-histamine receptor in the brain regions of epileptic and normal rats.

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Distribution of the H1 histamine receptor was studied in the brains of normal and epileptic rats (with convulsive, and/or non-convulsive seizures). Rats of the KM (with audiogenic seizures, N=7), WAG/Rij (with absence seizures [1], N=7), WAG/Rij-Auds (with both audiogenic and absence epilepsy, N=7) and Wistar (non-epileptic, N=7) strains were used in the present study.

Absence seizures are known to be frequent and spontaneous; to parallel the recurrent seizure state and avoid kindling conditions, the audiogenic seizures (Aud-s) were evoked in KM and WAG/Rij-Auds rats 3 weeks and 1 day prior to decapitation. After decapitation, the brains were removed, frozen in the liquid nitrogen and stored at –70C until 20mcm slices were cut. To assay the density of H1 histamine receptors, the slices were incubated with H3 - pyrilamine, as described before [2]; and apposed to 3H-sensitive films (3H-HyperfilmÒ, Amersham) at –20°C for 3 weeks, together with Amersham 3H Microscale Autoradiography StandardsÒ; 2 or 3 sections per rat per level were analysed. Optical densities were assessed by a computerised image analysis system within the following structures: the frontal cortex (motor and somatosensory regions), parietal cortex, n. caudatus (head and ventral part), caudoputamen, globus pallidus, n. accumbens (shell and core), ventroposterior thalamus, hippocampus (CA1, CA2, CA3 fields a

Comparisons made between the strains revealed, that in WAG/Rij rats H1 receptors were decreased in the S. nigra pars compacta [$F(1,25)=5.9$, $p<0.02$] and the dentate gyrus [$F(1,91)=4.8$, $p<.03$], but increased in the frontal motor cortex [$F(1,42)=4.4$, $p<0.4$]; as compared to those of Wistar and KM strains. Rats with AS (KM and WAG/Rij-aud) had lower density of H1 receptors within the cerebellum [$F(1,41)=9.09$, $p<.004$; n. interpositus, lateral cerebellar n. pooled together], as compared to the rats without AS. H1 histamine receptors were elevated in the hypothalamus of KM rats, compared to others [$F(3,81)=4.84$ $p<.004$] . Epileptic rats (KM, WAG/Rij, WAG/Rij-as pooled together) had lower density of H1 receptors within the n. accumbens [$F(1,44)=4.2$, $p<.04$; core and shell pooled together]. Comparisons, made within anatomical regions (all rat groups analyzed together), revealed that the forebrain structures generally had lower amount of H1 histamine receptors, than the hindbrain and brainstem regions. Among the hippocampal subregions, the dentate gyrus was the richest with H1 receptors [$F(1,91)=22.1$, $p<.000009$] as compared to others; among the dopaminergic brainstem nuclei, S. nigra pars compacta had higher H1 receptors density than ventral tegmental area [$F(1,142)= 13.5$, $p<.003$], and also higher than S. nigra pars reticulata [$F(1, 142)=40.9$, $p<.00000$]. The level of H1 receptors was higher in the n. accumbens shell, as compared to the n. accumbens core [$F(3,44)=6.6$, $p<0.02$]. Within the hypothalamus, the highest level of H1 receptors was found in the n. arcuatus [$F(1,81)=44.9$, $p<.00000$] and the lowest one in the posterior hypothalamus [$F (1,81)=33.6$, $p<.00000$].

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Comparing two different methods on two isolated preparations for estimation of antagonist potency

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One of the ways to determine the potency of an antagonist is to measure and calculate its pA2 value.

In our previous experiments, we used an H2 antagonist, famotidine, and determined its potency on two isolated organs, both containing H2 receptors: a superfused patch of guinea pig stomach and guinea pig right atrium. We used two different approaches, the method according to Schild and the method of Calderone.

In the present work, we used another H2 receptor antagonist – tiotidine – on both isolated preparations and with use of both above mentioned methods. It is not clear whether tiotidine is competitive or non-competitive antagonist. Our purpose was thus to discover, whether the values of pA2 and slopes of the corresponding lines, depend on the chosen method and isolated organ and to gather more data about the nature of tiotidine.

By Schild analysis on the stomach preparation, the slope didn't differ from unity (0.11 – 1.05), neither did by Calderone analysis (0.23 – 1.19). The experiments on the right atrium, furnished also the slopes, which didn't differ from unity, neither by Schild analysis (0.99 – 2.99) nor by Calderone analysis (0.83 – 1.09).

Concerning the pA2 values, those obtained on the stomach by each method didn't differ significantly: Schild method gave pA2 value of 7.44 and Calderone method gave the same value. The values of pA2 on the atrium were also not statistically different: Schild method: 7.43 and Calderone method: 7.15.

These results lead us to the following conclusions: no matter which of the two methods we choose, there is no statistically significant difference between the pA2 values, obtained on the same isolated organ. Also the choice of the isolated organ did not influence the pA2 value. The slopes are equal to unity, independently of the chosen method, indicating competitive antagonism, at least at the concentrations used.

Mepyramine, an H1 inverse agonist, binds preferentially to a G protein coupled form of the receptor and sequesters G Protein

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Knowing the importance proper characterization of ligand molecular mechanisms of action has for research and pharmacological uses, the aim of the present work was to investigate the processes mepyramine could use to exert its negative efficacy at the guinea pig H1 receptor stably expressed in Chinese hamster ovary (CHO-K1) cells. We found that this ligand binds with high affinity to a G protein-coupled form of the receptor and, as predicted by the Cubic Ternary Complex Model of receptor occupancy, mepyramine acts as an inverse agonist by promoting a G protein-coupled inactive state of the H1 receptor, interfering with the Gq-mediated signaling of the endogenously expressed ATP receptor. This effect was specifically neutralized by Gαq overexpression, indicating that mepyramine is able to reduce Gαq availability for other non-related receptors associated with the same signaling pathway. Finally, we found a loss of mepyramine efficacy in decreasing basal levels at high Gαq expression levels, which can be theoretically explained in terms of high H1 receptor constitutive activity.

The whole of the present work sheds new light into the understanding of H1 receptor pharmacology and the mechanisms inverse agonists could use to exert their observed negative efficacy.

FM and CPF contributed equally to this work.

Antiplatelet and antiphagocyte activity of H_1-antihistamines.

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Pharmacological activity of antihistamines strongly depends on subtype receptor interactions. Due to their cationic amphiphilic structure many antihistamines exert a variety of activities, which might result in beneficial side effects. In this study we compared the effect of two H_1-antihistamines, Dithiaden®(DIT-1st generation) and Loratadin®(LOR-2nd generation), on human blood platelet(BP) aggregation and polymorphonuclear leukocyte (PMNL) phagocytic activity in vitro. Platelet aggregation was measured turbidimetrically (aggregometry), phagocyte activity by means of stimulated, luminol-enhanced chemiluminescence (CL). DIT but not LOR dose-dependently inhibited platelet rich plasma aggregation induced with ADP and adrenaline. In isolated platelets stimulated with thrombin and A23187, DIT was 2.5 times more effective in comparison with LOR. On the other hand LOR was very effective in inhibiting PMA-stimulated platelets, starting at 10 μmol/L concentration. Compared with DIT, LOR in concentrations of 50 and 100 μmol/L was significantly more active on decreasing the CL in cell free system. In whole blood and on isolated PMNL DIT respectively was 2 and 5 times more effective, respectively in decreasing stimulated (opsonised zymosan) CL.

Antiplatelet and antiphagocyte effect of H_1-antihistamines DIT and LOR might contribute to their beneficial side effects. These concern the combination of specific antihistamine activity with antiplatelet and antiphagocyte-antioxidative effects. Results in vitro should be compared with studies on volunteers and patients.

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Notes

The curative effect of histamine on cutaneous wound healing process

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The skin is a crucial organ for protection of the body from the outer environment. The study of the skin wound healing processes, therefore, is very important medical issue. We assume that histamine would contribute in the wound healing reaction, because it has been shown to contribute to allergic and inflammatory reactions. Histidine decarboxylase gene-knockout (-/-) mice basically with no histamine in their organs are used to assess the effect of the presence of histamine in the skin wound healing. The 5 mm diameter round punched-out skin wound was prepared on the back of +/+ and -/- mice and we measured for their wound area every day. -/- mice resulted in the delay of wound healing and that exogenously administered histamine recovered this wound healing delay. The overproduction of histamine in the HDC gene-transgenic mice accelerated the healing compared to the wild type mice. Therefore, histamine is the positive accelerator of cutaneous wound healing. Macrophage infiltration and angiogenesis at the wound edge were only observed in +/+ mice but not in -/- mice. The amount of bFGF was higher in HDC (+/+) mice especially on the 1st day of wound healing compared to those in HDC (-/-) mice. SU 5402, a specific antagonist of bFGF, suppressed the angiogenesis and wound healing in HDC (+/+) mice but not in HDC (-/-) mice. On the other hand, PTK 787, a specific antagonist of VEGF had only minor effect on angiogenesis and wound healing. From these observations, the positive wound healing accelerating activity of histamine were mediated by the activity of bFGF which lead to angiogenesis in the wound healing process.

The effects of H1 antagonists on the methamphetamine-induced psychomotor activation in rats: The correlation to tissue methamphetamine concentrations

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Methamphetamine (MAP) is a potent psychomotor stimulant, while the first generation H1 antagonists are well known to cause sedation in man. Several studies demonstrated that the first generation H1 antagonists potentiated the MAP-induced psychomotor activation and two possible mechanisms had been postulated. One is the blockade of the central histaminergic neuron system and the other is the inhibition of dopamine reuptake. However, the exact mechanisms are still controversial. In this study, we examined the effects of H1 antagonists (d-and l-chlorpheniramine, pyrilamine, and ebastine) on MAP (3 mg/kg)- induced psychomotor activation using both prepulse inhibition (PPI) of startle responses and locomotor activity tests. Locomotor activity was measured every 10 min for 6 hours after drug administration. PPI was measured at 30 min and 180 min after drug administration. We also measured both serum and brain tissue concentrations of MAP in rats which received MAP in combination with d-chlorpheniramine (7.5 mg/kg) in order to examine whether H1 antagonists can change the metabolism of MAP. Serum and brain tissue MAP concentrations were measured in another rats at 180 min after drug administration. We found that the first generation H1 antagonists, in particular chlorpheniramine, significantly potentiated the MAP-induced psychomotor activation in the behavioral tests and that the serum and brain tissue concentration of MAP in rats which received MAP in combination with d-chlorpheniramine (7.5 mg/kg) was about eight and five times, respectively, higher than that of MAP alone. These results suggest that the first generation H1 antagonists significantly enhance the MAP-induced psychomotor activation mainly by altering the pharmacokinetics of MAP. However, we could not exclude another mechanisms such as the blockade of central histaminergic neuron system and the inhibition of dopamine reuptake. Therefore, we should consider not only the central effects, but also the peripheral effects of H1 antagonists.

Association of altered brain histaminergic system and related genes with addictive behaviour and psychiatric diseases

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The brain histamine (HA) system has been implicated in rat models of alcoholism and neuropsychiatric diseases. To investigate this in humans, H3 receptor binding using [^{3}H]-N$^\alpha$-methylhistamine as a ligand in autoradiography was carried out on the postmortem brain sections obtained from 15 schizophrenic patients and 15 controls. The binding was higher in the frontal cortex of schizophrenic samples compared to that of controls, particularly in the patients treated by atypical antipsychotics. The average H3 binding level in the superior temporal cortex of non-medicated schizophrenics was lower than that in the normal controls. However, the binding was significantly higher in the temporal cortex of patients treated by atypical antipsychotics compared to that in the non-medicated patients. The results suggest that atypical antipsychotics may elevate the H3 binding in the frontal and temporal cortices of schizophrenic patients. Moreover, the generally higher H3 binding in the schizophrenic frontal cortex suggests H3 receptor may be involved in the pathology of schizophrenia.

The study sample consisted of Finnish men aged 18-60 years and categorized as currently (n=75) and formerly dependent (n=29), and never alcohol-dependent subjects (n=110). Alcoholism was assessed using DSM-III-R and ICD-10 criteria. Genotyping was performed by either PCR following direct sequencing (h3rG3605A, h3rG3623A) or restriction fragment length polymorphisms (h3rA(-2807)G, h3rC2998T, h3rA5893G, hmtC314T).

None of the SNPs in H3R were associated with alcoholism. A weak but significant association (p=0.044) was detected between the functional C314T polymorphism in HMT and alcohol dependence. The C314T transition in HMT results in a Thr105Ile change in encoded amino acid, and this allelic variant displays significantly lower activity and thermal stability *in vivo* and *in vitro*. The lower pace of HA inactivation caused by a low activity HMT allele could contribute to high brain HA levels. Due to the relatively small sample size and the limitations of association analysis, replication studies as well as studies of HMT activity in alcoholic subjects are warranted.

Histamine Deficiency alters the testosterone production of Leydig cells in primary culture

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Earlier we performed studies on the steroidogenesis of HDC knockout mice. Our results showed that in males endogenous histamine deficiency interferes with normal gonadal development and has an impact on adult steroidogenic activity (1).To elucidate the role of histamine in the steroidogenesis of Leydig cells, we carried out experiments on primary Leydig cell cultures. Leydig cells were obtained from adult Balb c wild type and Balb c HDC knockout mice testes. The basal and hCG-induced testosterone production of the Leydig cells were measured with ELISA. Also RNA was extracted from the cells to compare the expression of steroidogenic enzymes and of histamine receptors with Real time PCR method.We found lower testosterone production of both basal and hCG-induced Leydig cells of knockout mice. Their response to hCG stimulation was significantly, twofold stronger than the response of wild type group. The distinct difference that can be observed between the cultured Leydig cells of wild type and knockout mice in the way they respond to hCG stimulation definitely reflects some influence of histamine on the steroidogenic activity of these cells.The fact that histamine has an impact on testosterone synthesis is in concordence with our earlier in vivo results, even though the tendency seems to be opposite. In our earlier studies we found that adult knockout mice had higher androgen levels than wild type ones, independent on GnRH expression. Differences of H1 and H2 receptor expressions between an in vivo system - the whole testis, as an organ - and in vitro cultured cells may serve as a possible explanation. Furthermore, paracrine factors play important regulatory role in the steroidogenesis of in vivo Leydig cells. To understand the whole mechanism, further experiments are needed.

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Cinnamic Amides of (*S*)-2-(Aminomethyl)pyrrolidines – Discovery and SAR of the Potent H3-Antagonist NNC 0038-0000-1202

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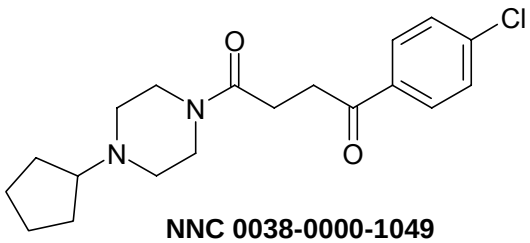
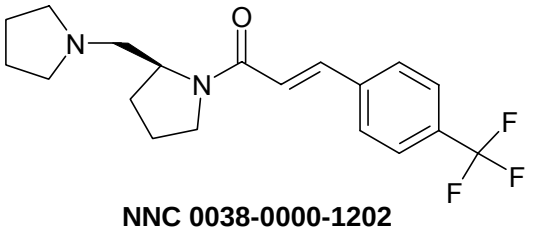
The successful discovery of monoamides of piperazine, exemplified by NNC 0038-0000-1049 [1], as selective and potent H3-antagonists resulted in the continued search for structurally related histamine H3-ligands with superior drug-like properties. Replacement of the piperazine ring in NNC 0038-0000-1049 with other constrained diamines led to the discovery of cinnamic amides of (*S*)-2-(aminomethyl)pyrrolidines as a new class of imidazole free H3-antagonists. Variation of the aromatic substitution pattern as well as structure and chirality of the diamine moiety gave rise to a series of compounds in which NNC 0038-0000-1202, (E)-1-((S)-2-((pyrrolidin-1-yl)methyl)pyrrolidin-1-yl)-3-(4-(trifluoromethyl)phenyl)propenone, was found to be a potent histamine antagonist with excellent selectivity for the H3-receptor.



NNC 0038-0000-1049



NNC 0038-0000-1202

The synthesis, in vitro binding characteristics and PK-data of NNC 0038-0000-1202 and its congeners will be shown. Most of the cinnamides are readily prepared in few steps with starting material from commercial sources. The SAR of these compounds with respect to H3-binding will be discussed.

In conclusion, a new class of drug-like, selective and potent H3-antagonists has been discovered. The compound NNC 0038-0000-1202 has been selected for in vivo characterization in different species.

[1] F. Zaragoza et al., 32th EHRSMeeting, Noordwijkerhout, May 2003.

Characterization of functional polymorphisms of the human diamine oxidase gene

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Diamine oxidase (DAO) catalyzes one of two alternative pathways of histamine inactivation and is likely responsible for scavenging extracellular histamine after mediator release. Reduced histamine degradation capacity due to low DAO activity has been implicated in the pathogenesis of inflammatory disorders especially in the intestine where DAO is prominently expressed. Previously we analyzed genetic variations of the human DAO gene to identify possible markers associated with diminished DAO activity and disease predisposition. However, none of these DAO gene polymorphisms tested turned out to have clear phenotypic consequences. Therefore, we screened for new polymorphisms that either lead to amino acid substitutions in the protein or alter regulatory elements in the gene promoter. To this end we completely sequenced the DAO genes of individuals that exhibited low intestinal DAO activity. This search identified in addition to the previously characterized C995T ($Ser^{332}Phe$) polymorphism in exon 2 a C47T ($Thr^{16}Met$) polymorphism in the same exon, a C4106G ($His^{646}Asp$) polymorphism in exon 4, and a G-4586T change in the promoter region. Although only a small number of samples have been analyzed so far, it appears that the ^{332}Phe variant is associated with a lower and the ^{16}Met and ^{646}Asp variants with higher intestinal DAO activity whereas the promoter variation has no phenotype. In vitro expression experiments and analysis of larger patient and control groups will have to confirm these findings and to evaluate a possible role of these genetic variants for disease progression.

Phenotypic profiling of experimental murine melanoma tumors with transgenically manipulated HDC expression

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Malignant melanoma represents a fairly dangerous skin neoplasia with continuously growing incidence in the industrialized world. In spite of the intensive research done on the mechanisms underlying melanoma progression, and the large body of experimental data reporting massive histamine secretion associated with these tumors, a detailed unifying concept explaining the exact role of histamine in this context is still unavailable.

In order to assess the possible impact of autonomous histamine secretion on melanoma progression we investigated the phenotypic changes caused by transgenic manipulation of the tumor histamine-synthetic activity in an experimental mouse melanoma model. B16-F10 mouse melanoma cells, being capable of giving rise to both primary skin tumors and lung metastases in syngeneic C57Bl/6 mice, were manipulated by stable transfection. Designated B16-F10–HDC.S, –HDC.A, and –HDC.M, three variants of the original cell line, harboring mammalian expression systems constitutively expressing the full-length sense HDC ORF, a partial antisense HDC sequence, and a mock transfection control, respectively, were subjected to a comprehensive phenotypic screening with respect to their progression profile. Comparative analysis of tumor growth rate, metastatic colony forming potential, and expression patterns of 20 different markers associated with proliferiation, angiogenesis, invasivity, metastatic potential, local and systemic immonomodulation were carried out using RNase Protecion Assay and, in some cases, by Western blot.

Regarding tumor growth it could be shown that forced expression of HDC radically accelerates the growth of melanoma tumors arising from B16-F10 cells inoculated in the skin. Metastatic potential, measured by numbers of lung lesions appearing after introduction of B16-F10 cells in the tail vein, exhibited a significant decrease in the presence of HDC antisense mRNA. Finally, results from tumor progression profiling demonstrated, that several markers associated with invasivity (MMP-2), metastatic potential (rho-C) and histamine signaling (H2 receptor) are significantly affected by alterations in the intensity of histamine secretion.

The influence of amitriptyline and sertraline in vitro on the kinetics of exogenous histamine in cat blood

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The treatment of depression in clinical practice is based on the use of antidepressants with different mechanisms of action. Studies in the field of histamine pharmacokinetics have shown that antidepressants modify the pharmacokinetics of histamine in cats and rats. They abolish the increase of histamine concentration in plasma after the injection of exogenous histamine. In order to determine whether the blood cells can significantly participate in the modification of exogenous histamine kinetics, *in vitro* tests were performed on cat blood. Histamine uptake and its metabolism were studied by following histamine and tele-methylhistamine (methylhistamine) concentrations in blood cells. Antidepressants, either amitriptyline or sertraline, were added to the buffer-suspension of blood cells or to the whole blood samples. After five-minute incubation time, histamine was added for another five minutes of incubation. Amitriptyline was used as a representative of tricyclic antidepressants, while sertraline as a selective serotonin reuptake inhibitor. The concentration of histamine in the samples was determined spectroflourometrically, while the concentration of methylhistamine in the whole blood or in the cells was determined with HPLC. The study confirmed the formation of methylhistamine in the blood cells; however, methylhistamine concentrations were about 40% lower in the cells treated with amitriptyline than in the samples without amitriptyline. Both amitriptyline and sertraline *in vitro* increased uptake of exogenous histamine in cat blood cells, amitriptyline by 55%, and sertraline by 60%. Moreover, measurements of histamine in platelet rich and poor plasma showed that platelets play an important role on increased histamine uptake. Our results suggest that blood cells participate in the modification of pharmacokinetics of histamine. It may also be concluded that antidepressants amitriptyline and sertraline influence the changes in the concentrations of histamine in feline blood cells by uptake mechanism and by inhibition of histamine biotransformation.

Body-weight lowering effects of the cinnamic amide, NNC 0038-0000-1202, a novel histamine H3 receptor antagonist, in obese rodents

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The cinnamic amide NNC 0038-0000-1202 ((E)-1-((S)-2-((Pyrrolidin-1-yl)methyl)pyrrolidin-1-yl)-3-(4-(trifluoromethyl)phenyl)propenone) is a novel, imidazole-free histamine H3 receptor antagonist and inverse agonist with a potency of ~5 nM at the human histamine H3 receptor and ~60 nM at the rat histamine H3 receptor. Receptor potencies were measured using a functional cAMP-assay on whole cells and a GTP□S-assay on membranes from HEK293 cells stably expressing the H3 receptors from the two species. A 75-receptor counterscreen (Cerep) revealed that NNC 0038-0000-1202 acts specifically on the H3 receptor. It showed affinities of >10000 nM to the H1, H2 and H4 receptors. The synthesis of NNC 0038-0000-1202 is described in a poster presented at this meeting (B. Peschke et al.).

After peripheral administration, NNC 0038-0000-1202 penetrates the blood-brain-barrier and induces histamine release, as measured by microdialysis. This, together with the compound's pharmacokinetic properties, suggested that it could be used as a model compound to investigate effects on food-intake and body-weight in a rat model of dietary-induced obesity.

In diet-induced obese, aged rats, peroral administration of NNC0038-0000-1202 at 5 mg/kg resulted in a reduction in food-intake. This reduction was associated with a significant, 5-7%, decrease in body-weight after 3 weeks compared to vehicle treated controls. There were no effects on either locomotor activity, pica behavior or conditioned taste aversion at this compound dose. Generally, small or no changes were seen in variables of blood chemistry. However, plasma □-hydroxybutyrate levels were significantly elevated and plasma triglycerides were decreased, also in relation to pair-fed controls, indicating a specific effect on fat metabolism.

In conclusion, the novel, potent and specific histamine H3 receptor antagonist NNC 0038-0000-1202 increases histamine-levels in the hypothalamus, decreases food-intake in a specific manner, and leads to a 5-7% weight loss after 3 week's oral treatment.

Can histamine be tasted in wine?

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Histamine is a widely discussed ingredient in wine and causes some undesirable adverse responses. The aim of the presented study is to assess whether histamine can be perceived by human senses in commercially available wine. Ten test samples were drawn from Spanish (red from the wood) and German (bottled white) wine cellars. Vintage, type of grapes, alcohol, and rest sugar concentrations were documented. Immediately after opening the wine bottles, samples were tested using standardized sensitivity assessment methods by 12 educated test persons. The reference was 0 10 mg/l histamine in normal tap water. Histamine was measured by fluorimetric detection after HPLC (detection limit: d.l. = 0.45 ng/ml). The mean histamine concentrations were 3 mg/l (range 0.945 5.03 mg/l) in the Spanish red wine and 0.01 mg/l (range d.l. 0.025 mg/l) in the German white wine. The mean threshold for histamine in normal tap water was 1 mg/l + 0.6 mg/l (SDM, n = 11; one test person was excluded after pseudoallergic symptoms and overreaction at low histamine concentrations). Sensitivity criteria like irritation at the deep throat (pharyngeal irritation), tingling tongue, swell at the mucous membranes in the mouth etc. were defined to be characteristic for the specific taste/response of histamine. A positive correlation was found between histamine concentration in wine and the sensitivity intensity (y = 0.738x 1.94, R2 = 0.691; ranking of intensity of histamine in 10 wine samples). As other ingredients such as alcohol, rest sugar etc. did not reveal any distinct interfering correlations it can be suggested that experienced wine test persons can identify elevated histamine concentrations in commercially available wine.

Molecular Dynamics Simulations of Histamine H3-Receptor/Ligand Complexes

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The human histamine H3 receptor (hH3R) belongs to the family A of G-protein coupled receptors (GPCRs) and is predominantly expressed in the CNS where it inhibits the release of histamine and other neurotransmitters upon activation. The great pharmaceutical importance is reflected in the large number of recent publications describing new potential ligands or new therapeutic applications that could be elucidated for this target. Homology models of proteins can effectively assist this drug discovery process as they offer the possibility to understand receptor-ligand interaction on an atomic level and can bring new impetus in lead-finding via database-screening methods or de-novo design strategies. The recent crystallisation of the bovine-rhodopsin structure [1] has revealed a suitable basis for the approach of comparative modelling and was used as a reference structure for the generation of a model of the hH3R in the presented work.

In order to find optimal parameters for the simulation of the receptor/ligand complexes we first carried out extensive calculations with the reference structure bovine rhodopsin testing the influence of the nature of the stabilizing solvent environment (CCl4/H2O, DPPC/H2O), truncation of N- or C-terminal endings, choice of different protonation states for selected residues in the protein interior and consideration of internal water molecules as resolved in [2] upon the overall protein stability. Through these calculations we were also able to narrow down the number of possible interhelical interactions given in [3] to a number of core contacts that could be observed during the simulation and that we could mostly map in the model of the hH3R as can be expected for proteins sharing the same 3D-structure and activation mechanism.

Using this acquired experience together with mutational data of family A GPCRs, a homology model of the hH3 receptor was generated that can be stably simulated in a DPPC/H2O environment. After assignment of favourable side chain-conformations with the program SCWRL a binding pocket could be mapped due to the presence of a free volume in the receptor interior spanning from helix 3 to helix 5. So far, H3 receptor/ligand complexes with antagonists of the imidazole- and non-imidazole class were simulated using the GROMOS [4] force field. The resulting geometry of the binding pocket was used for docking studies with the program FLEXX. We currently evaluate the specifity of the binding pocket for hH3 ligands and work on a QSAR model for hH3R ligands.

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Tandem mass spectroscopy as a new reliable and sensitive method for the detection of n-tele methylhistamine (MH) in urine

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N-tele methylhistamine (MH) has been found as an important and stable allergy mediator excreted in increased amounts into the urine during allergic and inflammatory reactions of the gastrointestinal tract. Since the RIA, commercially distributed by Pharmacia, has been taken off the market, tandem mass spectroscopy was evaluated for the detection of MH by comparison with the Pharmacia RIA.

Fourty urine samples from patients with suspected gastrointestinally mediated allergy were investigated in duplicate by tandem mass spectroscopy and the RIA. For tandem mass spectroscopy, 1ml urine was purified after addition of an internal standard (1-methylhistamin D3) and pH control by ion exchange. MH of the eluate was then quantitatively detected by tandem mass spectroscopy using multiple reaction monitoring and the ion pairs 126.1/109.2 and 129.1/112.2, respectively. The Pharmacia RIA was performed as recommended by the manufacturer.

The mean MH excretion by tandem mass spectroscopy and the RIA was 12.5 $\pm$ 8.5 and 13.0 $\pm$ 8.5 respectively. Tandem mass spectroscopy was found to yield a sensitivity of 2.6µg/l (API 365) and 0.5µg/l (API 3000), respectively, to detect MH with linearity from 7.8 to 2000µg/l, while the Pharmacia RIA showed a sensitivity of 2.0µg/l. There was a highly significant correlation between tandem mass spectroscopy and the RIA ($r = 0.94$, $p<0.05$). Tandem mass spectroscopy was found to show clearly lower intra- and interassay variation (3,2% and 4.1%, respectively) than the RIA (intraassay - 12.5% and interassay variation 14.5%).

The comparison of these two MH assays demonstrates that both methods detect urinary MH in a reliable and sensitive way in the µg concentration ranges. Tandem mass spectroscopy is recommended for future MH diagnostics in allergy patients, mastocytosis or inflammatory conditions, since it correlates very well the Pharmacia RIA, shows lower coefficients of variation and provides a more exact data quantification than the RIA.

Characterisation of four genes encoding porcine copper-containing amine oxidases

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Copper amine oxidases (AOCs) use molecular oxygen to oxidatively deaminate primary amines to the corresponding aldehydes, ammonia, and hydrogen peroxide. In mammals, members of the AOC enzyme family have been implicated primarily in the inactivation of biogenic amines but also in the process of lymphocyte adhesion. However, neither the number of homologs nor the exact function of individual enzymes has been elucidated. Using partial cDNA sequences amplified by PCR as probes, we cloned full-length cDNAs and genomic sequences encoding four different porcine AOC proteins consisting of ca. 750 amino acid residues. These AOC proteins include orthologs of the histamine inactivating enzyme diamine oxidase (DAO), the membrane-bound retina-specific amine oxidase (RAO), and vascular adhesion protein-1 (VAP-1), a peripheral plasma membrane protein present on endothelial cells that has been implicated in lymphocyte binding. The fourth AOC protein is a VAP-1 ortholog that has a signal peptide rather than a transmembrane domain at the N-terminus, is expressed primarily in the liver, and is likely responsible for the amine oxidase activity present in mammalian plasma. The polypeptide sequences of the AOC proteins and the gene structures are highly conserved, indicating the common evolutionary origin of these four AOC genes.

Epsilon subunit expression and propofol modulation of $GABA_A$ receptors in acutely isolated histaminergic neurons in the hypothalamus

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The histaminergic tuberomamillary nucleus (TM) is inhibited during sleep by gabaergic inputs from the ventrolateral preoptic area (VLPO) which is sleep active. The recently discovered central role of the TM in the sedative component of anaesthesia (1) may require specific neuronal responses depending on $GABA_A$ receptor (GABR) composition. Its epsilon (ε)-subunit is of particular interest, as it confers insensitivity to anaesthetics upon heterologous expression in artificial receptors. The functional role and pharmacological properties of *native* receptors containing the ε–subunit are unknown. We investigated them in acutely isolated hypothalamic neurons, immunopositive for the ε–subunit protein, with the help of whole-cell patch clamp recording combined with single-cell RT-PCR. The majority of neurons were identified as histaminergic (tuberomamillary, TM) neurons. The general anaesthetic propofol activated (> 6 μM) and potentiated (> 0.5 μM) the GABR in all TM neurons. A noise variance analysis of GABA-mediated currents enhanced by propofol demonstrated a significant difference between ε–positive and ε–negative neurons. In the former channel noise did not differ between control and potentiated responses, in the latter channel noise was decreased in the presence of propofol. Thus, either the propofol-mediated modulation of channel opening is different in GABR containing or lacking the ε–subunit, or, more likely, the contribution of the two populations of GABR, propofol-sensitive and propofol-insensitive, in the resulting whole-cell currents may be responsible for the observed phenomena. Epsilon-positive TM neurons are always GAD65-positive (2) and they contain vesicles with GABA. The TM neuron subpopulation with anaesthetic-insensitive $GABA_A$ receptors would be spared from inhibition during anaesthesia, they could enhance the inhibition of the ε–negative neighbour neurons by releasing GABA.

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Human H_3 histamine receptor isoforms can form homooligomers.

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The H_3 Histamine receptor subtype is expressed almost exclusively in the CNS where it acts as both an autoreceptor regulating the release of histamine itself, and a heteroreceptor modulating the release of many other important neurotransmitters, including dopamine, noradrenaline, serotonin and acetylcholine (Chazot & Hann, 2001). The H_3 receptor was cloned in 1999 (Lovenberg *et al*., 1999), which initiated a new era in histamine research. Pharmacological heterogeneity in H_3 receptors within and across species has long been recognised. The reasons for this heterogeneity are complex and not fully understood. In all species tested so far the full length H_3 receptor encodes a polypeptide of 445 amino acids (predicted polypeptide M_r 47,000). Shorter isoforms with deletions predominantly in the third intracellular loop domain have also been identified (predicted polypeptide M_r range 35,000-45,000). Note that these splicing events yield potentially different protein sequences in rodents and man. Little is known regarding the oligomeric structure and signalling properties of native H_3 receptors. In order to define the importance of H_3 receptor heterogeneity, specific immunological probes are required. Our laboratory has developed the first anti-H_3 receptor antibodies (Chazot *et al.,* 2001). The first two antibodies were raised against human H_3 receptor sequences, anti-H_3 (349-358) and anti-H_3 (175-187), and detected two specific immunoreactive protein species (M_r 68,000 and 93,000), both suppressed by prior incubation with respective peptide antigens, in many adult rat brain regions. Cross-linking experiments have indicated that these putative dimeric proteins can form disulphide-linked higher oligomers (Hann & Chazot, unpublished findings).

Recently, we have developed a third anti-H_3 antibody, which may prove to be the first immunological probe specific for the human H_3 445 isoform. A lipofectaminePLUS transfection method was used to transiently express human H_3 receptor clones in HEK 293 cells. FLAG-tagged hH_3 445, hH_3 365 and hH_3 329 were successfully expressed in HEK 293 cells, confirmed using an anti-FLAG antibody as the primary antibody in immunoblots of transfected cell homogenates. Using the same immunoblotting protocol the latest anti-H_3 antibody was strongly reactive against the full length clone with little or no reactivity against the 365 and 329 isoforms, respectively. Interestingly, the hH_3 445 isoform migrated as a monomeric M_r 47,000, as well as larger immunoreactive species consistent with putative dimeric, trimeric and tetrameric structures. Again, as with the native preparations, following cross-linking, higher oligomers were also observed. These data provide the first evidence that the H_3 receptor may form higher homooligomers. Immunohistochemical studies with the new anti-H_3 antibody, using wild-type and H_3-/- mice (TW Lovenberg, Johnson and Johnson, USA) demonstrated clear specific (ie. absent from H_3-/- brain) labelling of cortical laminae II, striatal and amydaloid complex, but little or no hippocampal labelling, a distribution pattern distinct from that reported for our original anti-H_3 (349-358 antibody). We are currently determining the isoform specificity of the new anti-H_3 antibody versus rodent isoforms.

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Characterization of the porcine diamine oxidase gene promoter

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Diamine oxidase (DAO) catalyzes the oxidative deamination of histamine and other diamines. The enzyme is a member of the class of copper containing amine oxidases that possess the active-site cofactor 2,4,5-trihydroxyphenylalanine quinone. In mammals, the major sites of DAO expression are intestine, kidney, and placenta. Recently we cloned and characterized the porcine DAO gene, which spans ca. 14 kbp and consists of five exons interrupted by four introns. Regulation of DAO gene expression was investigated by constructing DAO gene promoter fusions with firefly luciferase cDNA and analyzing luciferase activity after transfection of various cell lines. These studies revealed that a stretch of just 150 bp immediately upstream of the first small non-coding exon confers high-level expression in LLC-PK_1 porcine kidney epithelial cells and in HT29 human colon carcinoma cells. Deletion and mutation constructs identified several regulatory elements in the porcine DAO core promoter that were also found in the human DAO gene promoter. These include a glucocorticoid response element, three tandem Ap2 sites, and an E-pal motif that is flanked by Pu1 and Sp1 sites. Conservation of regulatory elements in the human and porcine DAO gene promoters forms the basis of the similar tissue-specific expression of DAO in both species. However, it is not clear at present why expression levels are much higher in the pig compared to man.

Further Hints for Protean Agonism at Histamine H_3 Receptors with Novel 4-(ω-(Alkyloxy)alkyl)-1*H*-imidazoles

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Numerous compound classes have been developed in the research field of histamine H_3 receptor ligands [1]. Despite high affinity and excellent selectivity of many lead structures special emphasis is actually put on the distinct pharmacological behaviour of the compounds. In a series of novel imidazole-containing antagonists for the histamine H_3 receptor we have developed unsymmetrical aliphatic ether derivatives containing the ω-(1*H*-imidazol-4-yl)alkyl scaffold. The compounds contain terminal alkyl, cycloalkyl or unsaturated moieties with or without heteroatoms or other functionalities. The majority of compounds displayed potencies in the low nanomolar concentration range when tested in vitro on rat synaptosomes, e.g. FUB 385 (4-(3-(3-cyclopentylpropyloxy)propyl)-1*H*-imidazole; K_i = 7 nM). FUB 465, 4-(3-(ethoxy)propyl)-1*H*-imidazole, was proven as a useful tool for the characterization of constitutive activity in rodents [2]. It shows high oral potency in mice (ED_{50} = 0.26 mg/kg). For selected compounds their influence on [^{35}S]GTPγS binding was assayed in HEK293 cell membranes expressing the human H_3 receptor. Here these compounds, which were shown to act as antagonists in rodents, showed partial agonism with intrinsic activities from 0.39 to 0.73. These distinct responses are further hints for protean agonism at histamine H_3 receptors in this class of compounds.

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Alpha2-Adrenergic Receptor-Mediated Presynaptic Inhibition of GABAergic IPSPs in Rat Histaminergic Neurons

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Nuclei of the brainstem involved in behavioral state control are mutually interconnected. Histaminergic neurons of the posterior hypothalamus receive inputs from brain stem noradrenergic cell groups as well as from the locus coeruleus. The role of adrenergic inputs in histaminergic function is unclear. We examined the actions of adrenergic agonists on histaminergic neurons of the tuberomamillary nucleus (TM) using electrophysiological methods in a brain slice preparation. Evoked GABAergic inhibitory postsynaptic potentials (IPSPs) in histaminergic neurons were reduced in amplitude following the application of norepinephrine (2-20 μM) or clonidine (10 μM) but were not affected by isoproterenol (10 μM). Norepinephrine application caused no changes in membrane properties of TM neurons. Responses to exogenously applied GABA were unaffected by adrenergic agonists. Clonidine reduced the frequency of spontaneous IPSPs, an action that was blocked by yohimbine. Norepinephrine did not alter the amplitude distribution of bicuculline-sensitive miniature inhibitory postsynaptic currents (mIPSCs). Thus, GABA release onto TM neurons is modulated presynaptically by adrenergic α_2-receptors. Inputs from noradrenergic neurons of the brainstem will reduce the inhibitory actions of GABAergic inputs resulting in disinhibition of histaminergic neurons.

Pharmacological studies on the role of reactive oxygen species in IgE-dependent histamine secretion from human basophils

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Activated mast cells and basophils are known to produce reactive oxygen species and certain antioxidants have been shown to inhibit mediator release from these cells. These observations suggest that intracellular free radicals, in particular superoxide, may also participate in cell signalling pathways which control mediator secretion from histamine-releasing cells. In the present study, we examined whether the principal enzymes implicated in the formation and degradation of reactive oxygen species play a major role in IgE-dependent human basophil histamine release. Two major sources of superoxide are xanthine oxidase and NADPH oxidase. However, neither xanthine oxidase inhibitors, allopurinol and oxypurinol (1-1000 μM), nor blockade of NADPH oxidase by diphenyleneiodonium (DPI) (0.01-100 μM) affected anti-IgE-induced histamine release from human basophils. Furthermore, modulation of NADPH oxidase via inhibition of free arachidonic acid production using $AACOCF_3$ (0.01-100 μM) also had no considerable inhibitory effect. Enzymatic catabolism of superoxide due to superoxide dismutase (10-1000 U/ml) and its membrane-permeable functional analogue, tempol (1 μM–10 mM), also failed to significantly affect histamine release. In contrast, catalase (10-1000 U/ml), which degrades H_2O_2, induced a dose-dependent potentiation of histamine release, which was not reversed by the addition of H_2O_2. We conclude that, whilst H_2O_2 and catalase affect basophil activation, intracellular oxygen radicals do not appear to modulate IgE-dependent basophil signalling.

Influence of histamine on the process of human trophoblast differentiation.

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In the process of normal placental implantation, spiral arteries infiltration with extravillous trophoblast is crucial. During this process multiple modifications in expression of trophoblast surface proteins occur. The cells of invasive trophoblast become alpha6-beta4 integrin negative and alpha5-beta1, alpha1-beta1 and alphaV-beta3 integrins positive. Poor invasion reported in preeclampsia may be a consequence of decreased expression of the alphaV-beta3 integrin (a vitronectin receptor). Our previous studies showed increased histamine concentration in the tissue of preeclamptic placentae, but its influence on trophoblast invasiveness is uncertain. The aim of the study was to examine the effect of histamine on alphaV-beta3 integrin expression in human term trophoblast cultured in vitro.

Material and methods
Six placentas from primigravidas were obtained and after enzymatic digestion trophoblast cells cultures were established using modified Kliman's method. Histamine (1 □mol/l) or natrium chloride (0,9%) were added daily into the vessels (culture I and culture II, respectively). On day 2, 3 and 4, cells were detached from vessels and integrin alphaV-beta3 mediated cell adhesion were measured with ELISA. The cultures were finished after 4 days. Statistical analysis of the results was performed using student t-test and the differences were deemed statistically significant if $p<0,05$.

Results
The integrin alphaV-beta3 mediated cell adhesion examined at day 2 was 11.9%± SD2,1% higher in culture I, without statistical significance, but at day 3 was 48.7%± SD1,2% higher and at day 4 was 50.2%± SD1,3% higher (both differences statistically significant) than in culture II (control).

Conclusions
Histamine stimulates alphaV-beta3 expression in trophoblast cells culture from term placentas obtained after uncomplicated pregnancies.

TGF-β1-induced attenuation of isolated placental arteries response to histamine is stronger in preeclampsia.

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Vascular response to histamine is related to the local nitric oxide (NO) production. Transforming growth factor-beta 1 (TGF-β1) down-regulates NO synthases in vascular smooth muscle cells as well as can stimulate the production of potent vasoconstrictors (e.g. endothelin). Thus, observed in preeclampsia, abnormal vascular tone and reactivity, leading to high impedance placental circulation may be related to TGF-β1. Available data about TGF-β1 concentrations in the serum and placental tissue in normal pregnancy and preeclampsia are contradictory.

We examined comparatively (normal pregnancy – group I versus preeclampsia – group II) the influence of TGF-β1 on histamine-induced relaxation of isolated placental arteries in vitro. Arteries have been isolated from tertiary branch after insertion of the umbilical vessels into placenta. Six normal and six preeclamptic placentas were obtained. Rings of arteries (*N*=24 in each group) were prepared at 37^0 C, plunged in aerated Hanks' balanced solution, mounted on a micrometric manipulator and connected to isometric force-displacement transducers. After pretreatment with TGF-β1 (250pM), isometric (active) tension of the isolated arteries was recorded as a parameter of the vascular response to histamine given in increasing doses (1nM–10μM). Histamine-induced vasorelaxation was significantly ($p < 0.05$) diminished in preeclampsia. Mean response in group II reached 71.22 ±6.13 (% ±SEM) of the value recorded for group I. The differences in vascular response to TGF-β1 or histamine given alone were not significant.

We concluded, that in preeclampsia, TGF-β1 down-regulates histamine-induced, endothelium-derived NO-mediated relaxation in placental arteries. The pathomechanism related to another cytokine receptors abnomalities should be considered.

Mast cell-derived VEGF and VEGF receptor type 1, 2, and 3 expression in human term trophoblast culture – influence of hypoxia.

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Mast cells (MC) synthetize, store and release vascular endothelial growth factor (VEGF). Hypoxia may stimulate degranulation of MC and increases local concentration of VEGF. VEGF-dependent angiogenesis is mediated through VEGF receptors.

The aim of the study was to examine the influence of hypoxia on MC degranulation and VEGF receptor type 1(VEGF-R1), 2(VEGF-R2), and 3(VEGF-R3) expression in cultured human trophoblast in vitro.

Placental samples were obtained during cesarean sections, terminating normal pregnancies (N=9). Cytotrophoblast cells were isolated using enzymatic digestion and resultant cell suspension was fractionated on a 5-70% Percoll gradient centrifugation. The cells were then co-cultured with the suspension of cord-blood derived MC under normoxic (20% O_2) conditions. After 5 days, the cultures were divided into normoxic (Culture I) and hypoxic – cultured under reduced oxygen tension (2% O_2, Culture II). In the control culture, isolated trophoblast has been cultured alone, both under normoxic and hypoxic conditions. After next 24 hours, quantitative immunohistochemistry was applied for VEGF receptors identification in formalin-fixed trophoblast cultures. Expression of VEGF-R1 and VEGF-R2 was significantly lowered ($p<0.05$) in Culture II and amounted [%,±SEM] 69.17±3.03 and 74.48±3.29 (respectively) of the reference values (Culture I). Significant differences in VEGF-R3 expression were not observed. In the control culture hypoxia increased VEGF-R1 and VEGF-R2 expressions.

The results suggest, that MC degranulation produces down-regulation of VEGF-R1 and VEGF-R2. Pathologic conditions related to increased number of placental MC (e.g. diabetes) may be associated with changed angiogenic response to hypoxia.

The effects of histamine on rheumatoid synovial fibroblasts in vitro: a comparison with human articular chondrocytes.

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Rheumatoid arthritis (RA) is a disease characterised by chronic inflammation and cartilage destruction, with degradation and loss of fibrillar type II cartilage collagen being a major feature. Osteoarthritis is characterised by degeneration and loss of articular cartilage; fibrillations of the articular surface, matrix depletion and cell clusters all reflect the aberrant behaviour of the resident chondrocytes. As yet the pathogenesis of both these diseases remains to be elucidated. However, common to both is the involvement of proinflammatory cytokines, such as TNF[alpha]z and matrix metalloproteinases (MMPs) in promoting the breakdown of the cartilage matrix. Recently we have demonstrated that human articular chondrocytes (HAC) in vitro respond to histamine by modification of their MMP production and an increased proliferative rate (1,2). The present study was designed to compare the effects of histamine on rheumatoid synovial fibroblasts (RSF) and HAC in vitro. Subconfluent cultures of both cell types were incubated with histamine (20[micro]M) for 24h; the subsequent conditioned medium was collected and assayed for MMPs-1, -3, -8, -13, TNF[alpha] and PGE2 by ELISA or Quantikine methodology. On incubation with histamine RSF showed increased production of MMP-1, MMP-3 and PGE2 (statistical significance being $p = 0.028$, 0.02 and 0.032, respectively, students t-test. However, MMP-8, MMP-13 and TNF[alpha] were not detected in the culture media. The results for RSF contrast with those for HAC, the latter showing increased MMP-3, MMP-13 and TNF[alpha] production in response to histamine (statistical significance, $p = 0.02$, 0.005 and 0.008 respectively, students t-test), but MMP-1 expression was unaffected. Our results show that histamine modifies the behaviour of both HAC and RSF in vitro, but different effects were observed for the production of specific MMPs and TNF[alpha] by the two cell types. Work supported by The Health Foundation, UK

Immunolocalisation of histamine H1 and H2 receptors in cells and tissues from osteoarthritic and rheumatoid joints.

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Histamine has a recognised role in allergic and inflammatory reactions and is reported to affect various cell types such as macrophages, epithelial cells, eosinophils and various subsets of T-cells. We have previously shown that human articular chondrocytes (HAC) and rheumatoid synovial fibroblasts (RSF) respond to histamine in vitro by increased cAMP, PGE2 and matrix metalloproteinase production. Such data suggest the expression of histamine receptors by HAC and RSF in vitro. This immunohistochemical study has examined H1 and H2 receptor expression by both cell types in vitro and in situ. Tissues from osteoarthritic and rheumatoid joints removed at joint replacement surgery were fixed in Carnoys fixative or 4% buffered formalin and processed to parafiin wax. 5Mm tissue sections were cut, dewaxed and immunostained for H1 and H2 receptors using polyclonal rabbit antibodies (Chemicon), followed by detection with biotinylated anti-rabbit secondary antibodies, and alkaline phosphatase(AP)-conjugated avidin-biotin. AP was visualised using New Fuchsin and sections were lightly counterstained using toluidine blue. HAC and RSF in culture were seeded onto 8-well chamber slides, fixed in 70% alcohol or 4% buffered formalin and immunostained for the receptors as described above. A variable proportion of chondrocytes in the superficial layer of different OA cartilage specimens were positive for both H1 and H2 histamine receptors; and a minority of chondrocytes stained positive in the intermediate and deep cartilage layers. In rheumatoid synovium positive immunostaining of fibroblastic cells as well as mast cells was observed. Of particular interest was the observation of enhanced immunostaining of histamine receptors in some regions of the rheumatoid lesion (cartilage/bone:pannus junction). For the HAC monolayer cultures approximately 70% were found to stain positive for both the H1 and H2 receptors. For the RSF in culture weak immunostaining for both H1 and H2 was noted in approximately 30% of the cells. This immunolocalisation study has demonstrated for the first time the expression of histamine receptors by chondrocytes and synovial cells, both in situ and in vitro.

Work supported by The Health Fundation, UK

Attenuation of Th1 Effector Cell Responses and Susceptibility to Experimental Allergic Encephalomyelitis in Histamine H_2 Receptor Knockout Mice is Due to Dysregulation of Cytokine Production by Antigen Presenting Cells

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Histamine, a biogenic amine with both neurotransmitter and vasoactive properties, is well-recognized as an immunomodulatory agent in allergic and inflammatory reactions. It also plays a regulatory role in the development of antigen-specific immune responses. $CD4^+$ T-cells from histamine H_1 receptor (H1R) deficient (H1RKO) mice produce significantly less IFNγ and more IL-4 in *in vitro* recall assays compared to wild-type controls. H1RKO mice are also less susceptible to acute early phase experimental allergic encephalomyelitis (EAE) indicating that H1R signaling in $CD4^+$ T-cells plays a central role in regulating pathogenic T-cell responses. In this study, we show that mice lacking histamine H_2 receptor (H2RKO) are similar to H1RKO mice in that they develop encephalitogen-specific T-cell responses as assessed by proliferation and IL-2 production and present with less severe acute early phase EAE. However, unlike T cells from H1RKO mice, which exhibit a strong Th2 bias, T-cells from H2RKO mice do not. Rather, they are uniquely characterized by a significant inhibition of Th1 effector cell responses. Given that both histamine and adjuvants such as pertussis toxin modulate antigen presenting cell (APC) maturation and function, including T-cell polarizing activity, we analyzed the cytokines/chemokines secreted by APCs from wild-type, H1RKO and H2RKO mice. Significant differences in cytokine/chemokine production by APCs from unimmunized and immunized mice were delineated. APCs from H2RKO mice produce significantly less IL-12 and IL-6 and markedly greater amounts of MCP-1 compared to wild-type and H1RKO mice. Since MCP-1 is known to inhibit IL-12 production, the failure of H2RKO mice to generate encephalitogenic Th1 effector cell responses is consistent with inhibition of negative regulation of MCP-1 secretion by H2R signaling in APCs.

Börje Uvnäs-Symposium

Antagonists of the histamine H4 receptor

RL Thurmond

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Histamine mediates its physiological function through binding to four known histamine receptors. Here we describe the discovery and SAR of the first selective antagonists of the histamine H4 receptor, the newest member of the histamine receptor family. These antagonists have high affinity of the human receptor with Ki values in the low nM range. There is at least 1000-fold selectivity over H1, H2 or H3 receptors and no cross-reactivity against 50 other targets. Compounds from this series have oral bioavailability in rats and dogs. The antagonists are able to blocks histamine-induced chemotaxis of mast cells both in vitro and in vivo. The antagonists have also been used to probe the role of the H4 receptor in chemotaxis and adhesion molecule upregulation in human eosinophils. Selective H4 receptor antagonists may have the potential to be useful in treating inflammation in humans.

Effect of H_3 receptor ligands on ethanol induced damage in rat gastric mucosal cells

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(R)-α-methylhistamine and FUB 407 [(3-1*H*-imidazol-4-yl)propyl 3,3-dimethylbutylether], agonists of histamine H_3 receptors, protect the rat gastric mucosa against differently acting damaging agents. The hypothesis that these compounds might exert direct cellular protection was presently investigated. A mixed population of gastric mucosal cells (GMC) from rat stomach was obtained by enzymatic digestion. Cell injury was induced by treatment with the medium containing 15% ethanol for 5 min and the cell viability was assessed by trypan blue (TB) exclusion and nuclear fluorescence induced by ethidium bromide (EB). Ligands for histamine receptors were added 60 min before ethanol challenge. Treatment with ethanol severely damaged GMC and cell viability was 39.5 ± 3.9% and to 43.1 ± 3.7%, as assessed by TB exclusion and EB fluorescence assay. Following a 60 min incubation period, (R)-α-methylhistamine increased cell viability to 49.4 ± 4.3% at 10^{-5} M, 50.5 ± 6.3% at 10^{-4} M and 52.7 ± 1.1% at 10^{-3} M, as assessed by TB exclusion and to 49.0 ± 10.9% at 10^{-5} M, 51.1 ± 7.9% at 10^{-4} M and 55.9 ± 4.1% at 10^{-3} M, as assessed by EB fluorescence. Despite the observed tendency for (R)-α-methylhistamine to increase cell viability, the values were statistically not different from those obtained in absence of (R)-α-methylhistamine preincubation. Following FUB 407 preincubation, cell viability was 49.1 ± 4.8% at 10^{-6} M, 50.5 ± 2.9% at 10^{-5} M, 50.7 ± 5.8% at 10^{-4} M by TB exclusion and 45.7 ± 3.8% at 10^{-6} M, 52.1 ± 3.8% at 10^{-5} M and 55.5 ± 7.2% at 10^{-4} M by EB fluorescence. The values did not reach statistical significance. The antagonists of H_1 (mepyramine), H_2 (famotidine) and H_3 (ciproxifan) receptors did not modify the damaging effect of ethanol. Present data suggest a minor role for a direct mechanism in the protective effect of H_3 receptor agonists.

Histaminergic modulation of the hippocampal and striatal EEG oscillatory profile in the behaving rat as revealed by Wavelet Feature Extraction

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It is well known that aminergic systems exert widespread adaptive neuromodulation. Collective neuronal activity is represented by oscillations of the local field potential at various frequencies. An unresolved problem is how network oscillations are coordinated in time and frequency domains by neuromodulatory systems. The electrophysiological effects of systemic injections (i.p.) of zolantidine, a H2-receptor antagonist, and ciproxifan, an H3-receptor antagonist, were measured in a pseudorandomised experiment in the behaving rat (n=6). The influence of these drugs on striatal and hippocampal (ventral CA1 area) EEG was evidenced by Wavelet Feature Extraction. Wavelet Feature Extraction is a promising new method with a good frequency and very high time resolution for electrophysiological signals; this method has recently been introduced and improved in our lab. Distributions and cross-correlations (msec to sec range of lags) of the EEG local power maxima are compared in the different pharmacological situations with a reference to vigilance states of an animal.

BÖRJE UVNÄS - A PIONEER IN MAST CELL RESEARCH
- the person and visionary pharmacologist

Börje Uvnäs, an honorary member of the European Histamine Research Society since 1991, died on November 5th 2003 at the age of 90. He joined the Histamine Club at the very beginning and has attended nearly every meeting since. He served as National Secretary for Sweden for many years and hosted the EHRS meeting in Stockholm in 1979.

Börje Uvnäs was a physiologist by training and was appointed professor of Physiology at the university of Lund in 1949 and professor of Pharmacology at the Karolinska Institutet in Stockholm from 1952 until he retired 1979. He was dean of the Medical Faculty for nearly 10 years, a member of the Nobel Committee for many years including chairman from 1973 to 1975 and also a member of the Nobel Foundation Board. He stimulated the development of the pharmacology discipline and the creation of subdisciplines such as neuropsychopharmacology and clinical pharmacology in Sweden. He played an important role in establishing the Swedish Committee on Evaluation of Adverse Reactions to Drugs as well as drug committees in Sweden.

Internationally, he made a great contribution to the separation of pharmacology from physiology by the establishment of a division of pharmacology within the International

Physiology Union, and was appointed general secretary of the Section on Experimental Pharmacology (SEPHAR) in 1966, president of the First International Pharmacological Meeting in Stockholm in 1961 and the first president of the newly founded International Union of Pharmacology (IUPHAR) from 1966 to 1972.

He worked internationally for many years with pharmacological and toxicological research and education and actively contributed to the foundation of the International Society on Toxicology in 1959, the European Society for the Study of Drug Toxicity in 1962, the Indian Pharmacological Society in 1969 and also the International Society for Biochemical Pharmacology in 1970. Börje Uvnäs was a member of the WHO Advisory Committee on Medical Research from 1970 to 1974, and president of the Federation of the European Pharmacological Societies from 1990 to 1992.

Even after his retirement in 1979 he was still very active. From 1969 and more than 30 years onwards, he was president of a Swedish Medical Research Foundation and managed to greatly increase capital funding, much to the benefit of many young researchers today. He was chief editor of Acta Physiologica Scandinavica from 1983 until 2000, that is up to the age of 87.

Börje Uvnäs had three lines of interests in medical research (1): Gastric secretion - starting with his thesis work and the proposal that vagal impulses released gastrin (2), the sympathetic vasodilator system (3) and mechanisms of storage and release of histamine and transmittor amines and peptides (4, 5).

Börje Uvnäs was a great leader and a visionary in pharmacology and international cooperation. He had more than 300 publications and was an honorary member of many societies and received many decorations and scientific awards.

For the past 40 years Börje shared his life with Ingrid, who accompanied Börje to all Histamine Meetings and several other scientific assemblies. Börje was passionate about our Society, extremely interested in its wellbeing and often wrote to the President after meetings to share his opinions about the good and bad points experienced. Several young scientists have been able to benefit from the excellent constructive advice that Börje offered, even at his last meeting in 2003. Börje Uvnäs was a dynamic leader whose enthusiasm for science was infectious. He had a wonderful sense of humour, was generous and warmhearted with an instinctive understanding of good science. He will be greatly missed.

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Anita Sydbom

Characterization of Fluorescent Ligands for the Histamine H_2 Receptor

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Fluorescent assays are increasingly applied in a broad spectrum of scientific disciplines among which analytical chemistry, molecular- and cellular biology, drug discovery and pharmacology. In pharmacology, the ideal fluorescent ligand would allow the implementation of real-time, non-destructive approaches to study trafficking, quantity and binding properties of receptors in microscopical, microplate-reader and on-line detection formats. This not only requires hydrophilic, stable, non toxic and receptor selective ligands with an affinity at nanomolar concentration, but they should also possess a high fluorescence intensity under physiological conditions as present in cell culture media. In addition, excitation and emission spectra should be in the long wavelength-area and non-overlapping. For some G-protein coupled receptors (GPCRs), like adrenergic and 5HT receptors, fluorescent ligands have been developed that meet most of these criteria. For other GPCRs, like the histamine receptors, however, the development of fluorescent ligands has only just started. The synthesis and pA_2 values of fluorescent H_1- and H_2 receptor ligands were recently reported by L. Li and collegues in the group of Buschauer (Bioorg. Med. Chem Lett, 13, 2003 pp 1245 and pp 1717 for H_1 and H_2 respectively). However, the application of fluorescent histaminergic ligands in fluorescent assays has not been described. Therefore, we set out to evaluate the fluorescence characteristics and to find applications in fluorescence based assays on new fluorescent high affinity histamine H_2 receptor ligands. The fluorescent compounds are aminopotentidine (APT) derivatives containing either dansylamido, 7-nitrobenzoxadiazoleamino, 1-cyanoiso-indole, N-methylanthranilamido, 1-cyano-indolizine-2-carboxamido, ethyl indolizine-2-carboxylate-1-carboxamido or dimethylbodipypropionamido groups as fluorescent functionalities. The histamine H_2 receptor affinity was evaluated using the H_2 antagonist [^{125}I]-APT radioligand binding assays on membrane extracts obtained from COS-7 cells transiently expressing the rat histamine H2 receptor. The compounds exhibited H_2 receptor affinities ranging in pK_i values from <4 to 8.9. Currently, we are evaluating the biological activity and the applications of the ligands in pharmacological assays based on fluorescence detection.

Hiroshi Wada Memorial symposium

Obituary: Prof. Hiroshi Wada

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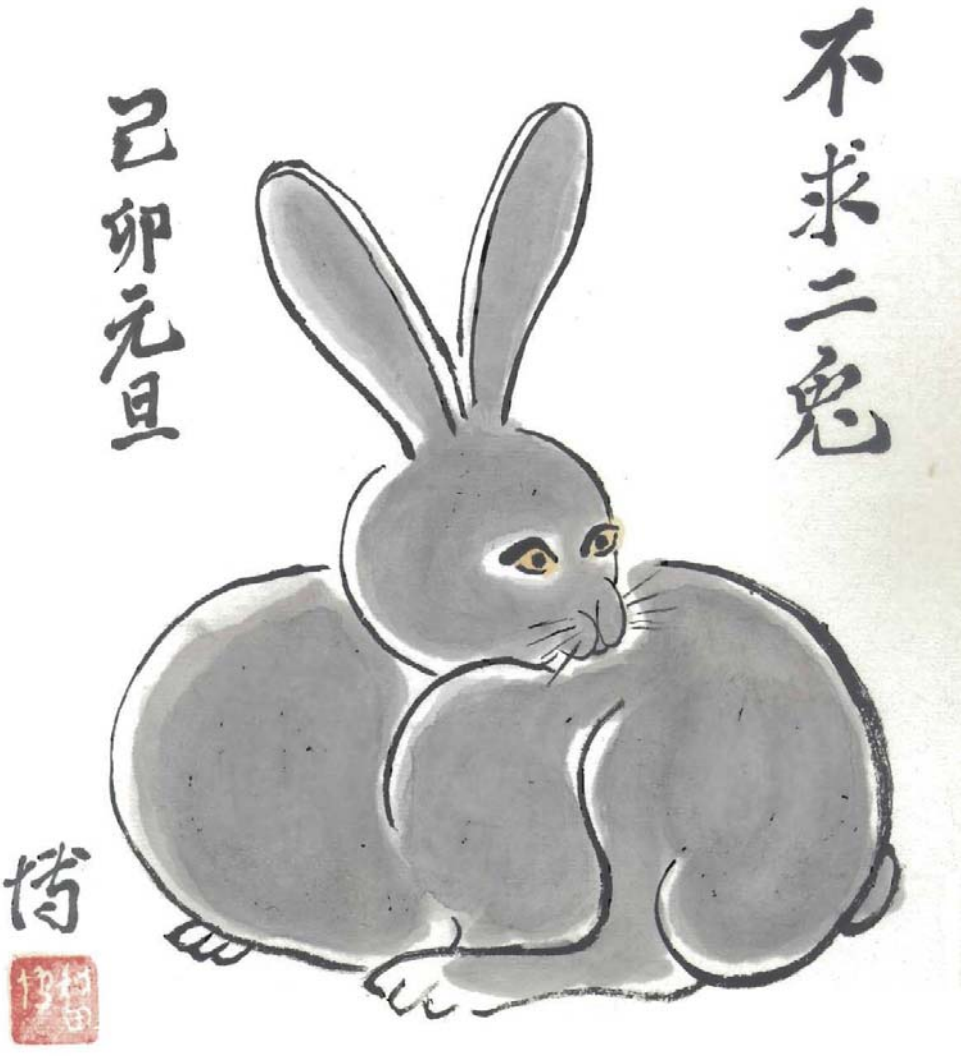
It is to our great regret that Prof. Hiroshi Wada died in Osaka, Japan, on June 21, 2003. He was born in Osaka on July 18, 1928 and graduated from Osaka University School of Medicine in 1953. He worked in Department of Biochemistry of the same school from 1954 to 1971, was promoted to Prof. of Pharmacology in 1972, and retired in 1992. After retirement he continued his scientific activity with lectures in public and in medical schools. Thus, he spent most of his life in Osaka except for a few years stay as a postdoctoral fellow in Prof. Esmond E. Snell's laboratory, Department of Biochemistry, University of California, Berkeley, USA. In biochemistry, he studied on pyridoxal enzymes such as tryptophanase and aspartate aminotranferase (GOT) isozymes. In pharmacology, he studied on biogenic amines, particularly histamine.

In 1984, he identified the location and distribution of histamine in the brain, which had not been clear at that time, by immunohistochemistry using antibody against histidine decarboxylase (HDC, a histamine-forming enzyme). It is interesting that HDC is a pyridoxal phosphate; his interest has been in pyridoxal-enzymes through his career. It is also interesting that Pertti Panula and Harry Steinbusch, independently, obtained the same results with anti-histamine antibodies as a probe. Since then Prof. Wada studied functions of the histaminergic neuron system in the brain using various tools, such as FMH, a specific inhibitor of HDC, and W/Wv mice, mast-cell deficient mice, with many young colleagues. It should be mentioned that 8 of them became professors of pharmacology and some of them are still working on histamine in their laboratories all over Japan. Furthermore, he cloned the histamine H1 receptor and clarified the distribution of H2 and H1 receptors in Type I and II astrocytes. His main interest was the circadian rhythm and he showed a beautiful correlation of the hypothalamic histamine content and spontaneous locomotor activity in rats. After retirement, he enjoyed recent developments on the role of histamine neurons in awake-sleep mechanism.

Prof. Wada was loved by all the people who met him and experienced his open-minded generosity. Hiroshi Wada was also a distinguished GO-player and a wonderful artist. We were planning to invite him to give us his memories of the histamine story and his advice for future development. He passed away but his creative influence on our field continues and he is gratefully remembered by all of us.

Prof. Wada's achievements were reviewed on the occasion of his retirement in 1992 by Takehiko Watanabe: Prof. Hiroshi Wada and his works --- A retrospective personal view. Ann. Psychiat., 3, 1-11(1992)



Do’nt want to catch two rabbits at once ! Drawing by Hiroshi Wada

Histamine elicits neuronal excitatory response of red nucleus in the rat via H_2 receptors *in vitro*

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Our previous studies revealed that histamine exerts an excitatory effect on cerebellar cortical Purkinje cells and interpositus nuclear cells. It is interesting that like the cerebellum, the red nucleus (RN) also receives afferent fibers from the hypothalamus and expresses histamine H_2 receptors. By using brain slice preparations, we investigated the effect of histamine on neuronal firing of the RN. Perfusing slices with histamine (1-100 μM) produced an excitatory response in rat rubral neurons (118/132, 89.4%). The histamine-induced excitation was not blocked by low-Ca^{2+}/high-Mg^{2+} medium (n=10), suggesting a direct postsynaptic action of the amine. The histamine H_2 receptor antagonist ranitidine effectively blocked the excitatory response (n=26), but the H_1 receptor antagonist triprolidine did not (n=24). The excitatory effect of histamine could be mimicked by the H_2 receptor agonist dimaprit (n=24), and the dimaprit-elicited excitation of rubral neurons could be blocked by ranitidine (n=16), but not by triprolidine (n=9). In addition, the H_1 receptor agonist 2-pyridylethylamine did not elicit any response in rubral neurons (n=12). Furthermore, the cells of both the magnocellular and parvocellular parts of the RN showed a unique excitatory response to the stimulation by histamine and dimaprit but no response to 2-pyridylethylamine, and the histamine- and dimaprit-induced excitation could be blocked by ranitidine, but not by triprolidine.

These results indicate that histamine excites RN neurons through H_2 receptors. Considering the fact that the RN is a target of cerebellar output, we suggest that the histaminergic fibers arisen from the hypothalamus may parallelly innervate, at the two hierarchies of the cerebello-rubro-spinal system, the cerebellum and RN and may modulate the sensorimotor integration through this circuit. (Supported by grants 30070250, 30370462 and 30318004 from the National Natural Science Foundation of China, RFDP grant 20010284021 from the State Educational Ministry of China and grant BK2002083 from the Natural Science Foundation of Jiangsu Province, China)

Histamine modulation of neuronal activity of rat cerebellar cortical Purkinje cells and deep nuclear cells *in vitro*

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Some studies have demonstrated that histamine is a candidate for the neurotransmitters used by the direct hypothalamocerebellar pathways and revealed the presence of histamine H_1, H_2 and H_3 receptors in cerebellar cortex and deep cerebellar nuclei of the rat. Although the action of histamine as a neurotransmitter in some brain areas has been widely evaluated, studies regarding its effect on neuronal activity of the cerebellum are still limited. By using cerebellar slice preparations, we investigated the effects of histamine on neuronal activities of the rat cerebellar Purkinje cells and interpositus nuclear cells. Perfusing slices with histamine (1-100 μM) produced, in the cerebellar cortical and deep nuclear neurons, an excitatory response in a dose related manner. The histamine-induced excitation was not blocked by low-Ca^{2+}/high-Mg^{2+} medium, indicating a direct postsynaptic action of the amine on the cells. The histamine H_2 receptor antagonist ranitidine effectively blocked the excitatory response, but the H_1 receptor antagonist triprolidine did not. The excitatory effect of histamine on the cerebellar neurons could be mimicked by the H_2 receptor agonist dimaprit, and the dimaprit-elicited excitation could be antagonized by ranitidine, but not by triprolidine. Furthermore, the H_1 receptor agonists betahistine, 2-thiazolylethylamine or 2-pyridylethylamine could not elicit any response in cerebellar neurons.

These results reveal that histamine excites cerebellar Purkinje and interpositus nuclear cells via H_2 receptors and suggest that the hypothalamocerebellar histaminergic fibers may play an important role in the function of the cerebellar neural network. In addition, because all of the cerebellar histaminergic fibers come from the hypothalamus, it also can be suggested that the functional actions of this pathway are closely related to the integration of somatic-visceral responses. (Supported by grants 30370462 and 30318004 from the National Natural Science Foundation of China, RFDP grant 20010284021 from the State Educational Ministry of China and grant BK2002083 from the Natural Science Foundation of Jiangsu Province, China)

Interleukin (IL)-9 increases the expression of several cytokines in activated mast cells and the IL9-induced IL-9 production is inhibited in mast cells of histamine-free transgenic mice

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RATIONALE:
Histamine and IL-9 are suspected to play an important role in the pathogenesis of asthmatic and allergic reactions. Transgenic mice overexpressing IL-9 either systemically or lung-specifically were shown to develop more severe asthmatic symptoms than their wild type littermates. Furthermore, IL-9 is known to induce the proliferation of mast cells resulting in mastocytosis during intestinal helminthic infections. Mast cells store large amount of histamine in their granules and are capable of producing different cytokines upon stimulation. In this study we investigated the IL-9-induced cytokine expression of activated mast cells and the differencies of this profile in the absence of histamine.
METHODS:
Mast cells were in vitro differentiated from bone marrows of wild type and histamine-free mice (which lack histidine decarboxylase, the only enzyme capable of forming histamine), these cultures were stimulated in different conditions and the cytokine expression profile was evaluated by RNase protection assay.
RESULTS:
In this study we show that mast cells stimulated by IL-9 and ionomycin or IL-9 and antigen specific IgE/antigen express several cytokines at mRNA level, among them IL-1β, IL-1Ra, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13 and MIF. Furthermore, both IL-9 and ionomycin are needed for the production of these cytokines in great quantities, which is mediated through the production of IL-1β. When examining the kinetics of cytokine expression, we could observe a decrease in the level of IL-4 and IL-13 and a marked increase in IL-9 mRNA over time. Histamine-free mast cells respond by a markedly decreased IL-9 expression to IL-9 + ionomycin or IgE/antigen stimulation.
CONCLUSION:
Our results show that IL-9 as a costimulator can activate the cytokine expression of mast cells. The IL-9-induced IL-9 production may result in a positive feedback loop and the lack of histamine disturbes this loop which may serve an explanation for the reduced asthmatic symptoms observed in histamine-free mice.

Double-blind, placebo-controlled oral provocation with histamine revealed placebo-associated histamine release in mastocytosis (M) but not in controls

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Mastocytosis (M) combines some types of diseases which are all characterized by proliferation and accumulation of mast cells in various tissues. In mastocytosis the number of mast cells is increased and mostly they are unripe, resulting in enhanced release of different mediators. Histamine intolerance may thus be a result of unregulated mediator release. Patients with M sometimes report on typical histamine symptoms because of only psychological/neurovegetative stimuli. To objectify this effect we carried out a standardized double-blind oral histamine provocation test in our intensive care unit. Two patients with M and three patients with gastrointestinally mediated allergy (as controls) were investigated. All patients received one placebo challenge (peppermint tea) and one histamine provocation (75mg, mixed in peppermint tea). Plasma histamine levels and clinical symptoms were followed at the time points –10, 0, 10, 20, 30 and 40 minutes. Histamine was measured by ELISA.

During provocation with histamine both groups reached similar plasma histamine levels within 30 minutes, in M 0.31 ng/ml x m² BSA $\pm$ 0.28 and in controls 0.30 ng/ml x m² BSA $\pm$ 0.28. Interestingly, a placebo-associated histamine release was observed only in M, which was also associated with clearly visible histamine induced symptoms (flush, tachycardia, agitation). This was associated with an objective measurable increase of the plasma histamine up to 0.87 ng/ml x m² BSA $\pm$ 0.69, whereas in controls plasma histamine remained in the normal range (up to 0.11 ng/ml x m² BSA $\pm$ 0.05).

Obviously in this test situation, patients with M show a placebo-associated or a neurotransmitter-, or psychologically induced histamine release, which may be a result of the proliferating unripe mast cells. In contrast, allergy patients as controls showed a strongly restricted mast cell secretion without a response to the given psychological stimulus “test situation on intensive care unit”.

Taurine chloramine modifies the development of adjuvant- induced arthritis

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We have previously shown that taurine chloramine (Tau-Cl), endogenous compound exerting some anti-inflammatory activities, modified the development of an acute inflammation. In the present study we evaluated the effect of taurine chloramine on adjuvant - induced arthritis (AA) in rats. This model resembles spontaneous arthritic disease in human. AA was induced in male Brown-Norway rats by injection 0.1 ml of ground heat-killed Mycobacterium tuberculosis suspended in incomplete Freunds adjuvant into the foodpad. Rats received taurine (Tau) (5ml 40mM i.p.) or Tau-Cl (5ml 10mM i.p.) once a day for 21 days. Treatment was started simultaneously with AA. The blood concentration of histamine, release of histamine from peritoneal mast cells and respiratory burst of neutrophils were estimated. Results of the study showed that the administration of Tau-Cl and Tau (less effective) during the chronic experimental inflammation :1. decreased of foodpad swelling 2. down-regulated elevated in AA histamine level in blood 3. normalized of neutrophils response to stimulation 4. diminished ability peritoneal mast cells to spontaneous and 48/80-stimulated release of histamine These results suggest that Tau-Cl is able to modulate induction of immune response at least in part by down- regulation the generation of inflammatory mediators (histamine, oxygen radicals species).

Is estimation of lymphocyte histamine content useful in the identification of nonresponders among rheumatoid arthritis (RA) patients treated with anti-TNF-alfa monoclonal antibodies?

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We have previously shown that total histamine content in lymphocytes from RA patients is significantly lower and these cells release more histamine spontaneously when compared to healthy individuals.Tumor necrosis factor-alfa (TNF-alfa) is a key cytokine in RA. TNF-alfa stimulates production of histamine by macrophages and T-cells. TNF-alfa bloking agent Infliximab (Remicade) is successfully used in the treatment of active RA however only 40-50% of patients are among the responders (ACR criteria). Objective. We determined the effect of treatment of RA patients with chimeric anti-tumor necrosis factor alfa antibody (Infliximab) on the secretion of histamine from peripheral blood mononuclear cells (PBMC), oxygen radicals production by PMNs and cartilage metabolism (cartilage oligomeric matrix protein- COMP and N-acetyl-beta-D-hexosaminidase activity - NAH-ase). Methods. 10 patients with refractory RA were treated with infliximab (3mg/kg) at weeks 0, 2, 6 and every 8 weeks in combination with methotrexate for 62 weeks. PBMC were stimulated with PHA and histamine was determined fluorimetrically. Chemiluminescence response of PMNs after zymosan stimulation was measured using Bio-Orbit luminometer. Serum COMP was measured by a sandwich ELISA and NAHase activity by colorimetric methods (p-nitrophenol/hr-1/ml-1). Results. Results of the study showed that anti-TNF-alfa long-term therapy caused in all patients: 1.normalization of neutrophils response to zymosan stimulation which was diminished in the course of RA. 2.decreased of NAHase activity (glicosidase involved in proteoglycan degradation)($p < 0.01$). 3.non-significant decrease of COMP levels (marker that reflects cartilage destruction process). 4. unchanged PHA stimulated release of histamine from PBMC. Two patients did not respond to anti-TNF-alfa therapy and both had 3 times lover PBMC histamine level when the therapy started. Conclusion. The estimation of PBMC histamine may be useful indicator in the response to the treatment. Further studies with more patients included are neccessary.

[^{11}C] Doxepin-PET Study of Histamine H_1 Receptors in Psychiatric Patients

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Increasing evidences have shown that the histaminergic neuron system is implicated in the pathophysiology of psychiatric disease. The aim of this study was to compare the distribution of histamine H_1 receptors between schizophrenic and depressive patients and normal human subjects in vivo using positron emission tomography (PET). Histamine H_1 receptor binding was measured in normal subjects and medicated schizophrenic and depressive patients by PET and□^{11}C□doxepin, a radioligand for the histamine H_1 receptor. The binding potential (BP=Bmax/Kd) of□^{11}C□doxepin for available brain histamine H_1 receptors was calculated by a graphical analysis on voxel-by-voxel basis, and compared between psychiatric patients and normal subjects using the resions of interest (ROIs) and the statistical parametrical mapping (SPM99). BP values for histamine H_1 receptors in the frontal and prefrontal cortices, and the cingulate gyrus were significantly lower among the psychiatric patients than among the control subjects. On the contrary, there were no areas of the brain where histamine H_1 receptors were significantly higher among the psychiatric patients than the control subjects. In addition, ROI-based analysis revealed that BP values were lower in the prefrontal cortex and cingulate gyrus of psychiatric patients. The results of our study suggest that the central histaminergic neuron system could be involved in the pathophysiology of psychiatric disease, although further studies are needed to confirm our results. The decrease of histamine H_1 receptors observed in the psychiatric patients in this study would be a consequence of downregulation caused by increased presynaptic histamine release from histamine neurons.

Complex pharmacology of GT-2331 in *in vitro* and *in vivo*

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Histamine H_3 receptors are constitutively active presynaptic receptors that regulate histamine release from synaptic terminals. As sometimes seen with other constitutively active receptors, the pharmacological blockade of H3 receptors can be complex and the results can be dependent on the assay system used. GT-2331, originally identified as an H3 antagonist, has been recently reported to behave as an agonist. Here we characterized in-depth the *in vitro* and *in vivo* profiles of GT-2331. ***In vitro*:** GT-2331 potently displaced [^{3}H]N^{3}MeHA binding to the cell membrane expressing recombinant rat H_3 receptors (rH3R) with an IC50 of 1.3 nM. Functionally, GT-2331 inhibited forskolin-induced cAMP production (IC50; 0.04 nM) in cells, and increased [^{35}S]GTPγS binding (EC50; 0.4 nM) in cell membranes, expressing recombinant rH3R in a dose-dependent manner. These results suggest that GT-2331 functions as an agonist. However, in [^{35}S]GTPγS binding assay using rat whole brain membranes, GT-2331 did not show any significant agonistic activity, although the control agonist, RαMeHA, significantly increased [^{35}S]GTPγS binding. ***In vivo*:** Intracerebroventricular (ICV) administration of Rα-MeHA (2.4 nmol/head), but not GT-2331 (up to 10 nmol/head or 10 mpk), potently elicited drinking behavior in rats. In contrast, icv or systemic administration of GT-2331 potently suppressed Rα-MeHA-induced drinking behavior, indicating that GT-2331 functions as an antagonist.

Conclusion: GT-2331 showed agonistic activities to the recombinant rH3R, but not to the native rH3R *in vitro*. It is generally known that the agonistic activity of small molecules in several *in vitro* assays depend on the expression levels of targeted receptors. Thus, the agonistic activity of GT-2331 might be due to superphysiological expression levels of recombinant receptors. The *in vivo* antagonistic profile of GT-2331 might be useful to address the physiological roles of H3 receptors.

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THE INTERNATIONAL ANTHEM OF
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CHORUS: For it´s mine, for it´s mine,
Decarboxylated Histidine.
We´ve extracted you and weighed you.
By the living gut assayed you.
But we´ve yet to find your function - **Histamine!**

1. We talk of toxicosis / migraine, shock or halitosis
Singing Histaminosis all the day.
Trauma, burns and inflammation / headache, pain and constipation,
Singing Histaminosis all the day.

2. You give asthmatic wheezes / the allergic sneezes, / Singing...
Though obscure as yet, the fact is / you´re involved in anaphylaxis, / Singing..

3. Since the time of Dale and Barger / your files are longer, larger / Singing...
The control of circulation / then gastric stimulation, / Singing...

CHORUS

4. Mast cells by the dozen / and basophils, your cousin, / Singing....
They come and they go / fluctuate to and fro, / Singing....

5. We heard a lot of groaning / from the upstart, Serotonin, / Singing....
Down with 5-hydroxytrypta / and up with good old hista, / Singing....

6. Each year we meet in May / to concentrate and play, / Singing....
What luck to have such friends / to cater for our trends, / Singing....

CHORUS

7. In nineteen seventy two / to Paris we all flew, / Singing....
Then Marburg upon Lahn / where Wilfried kept us calm, / Singing....

8. Copenhagen as next year / the Mermaid to cheer, / Singing....
In nineteen seventy five / Florence kept us alive, / Singing....

9. To Paris for the next / to hear a new text, / Singing....
In nineteen seventy seven / London, it was Heaven, / Singing....

CHORUS

10. Then Lodz with great care / we learned a lot there, / Singing....
In nineteen seventy nine / to Stockholm this time / Singing....

11. Then to Budapest we went / with Susan on the scent, / Singing....
West Germany again / for Hannover by name, / Singing....

12. In nineteen eighty two / to Bled we all flew, / Singing....
Then Brighton to the fore / with sea breezes by the shore, / Singing....

CHORUS

13. And in nineteen eighty four / back in Florence like before, / Singing....
Then in Aachen eighty five / Charlemagne became alive, / Singing....

14. Then in Odense in Spring / in the Castle we did sing, / Singing....
And then Czecho was the next / with our Rado at his best, / Singing....

15. G.B. West was then cheered / for the ten years we´d been steered, / Singing....
Let us sing this song together / Histamine will last forever, / Singing....

CHORUS

16. And in nineteen eighty nine / it was also fine,
There in Holland for the very first time.
To Kuopio in Finland / to the beautiful, but cold land,
we were watching the Finnish chopping wood.

17. Then to Marburg we returned / ninety one and also learned
that histamine in surgery´s not good.
The next year did we meet again / with Manuel in sunny Spain,
Singing ai, ai and olé all the way.

18. Then with Eddy on the Rhine, we had more beer than wine, / Singing....
To Zsuzsanna ninety four / we went back to Danube shore, / Singing....

CHORUS

19. Then with Igor ninety five / and the Volga was alive
And we entered the Russian Golden Ring.
In Antwerpen ninety six / Frans did show us a few tricks, / Singing....

20. To Sevilla, once again / we all met in lovely Spain, / Singing....
To Agnieszka ninety eight / back in Poland it was great, / Singing....

21. Then to Lyon ninety nine / and Histamine´s still mine / Singing....
New Millenium in Rome / Bruno made us all feel home / Singing....

CHORUS

22. Pertti took us on a boat / we and Histamine could float
So to Turku we came two thousand one.
Andras called two thousand two / and to Eger did we go
A Hungarian meeting once again

23. In the year two thousand three / we could lots of tulips see
Now Henk Timmerman was host in Amsterdam

Back to **Germany** next spring / and with **Helmut** did we sing
Singing Histaminosis all the day

CHORUS